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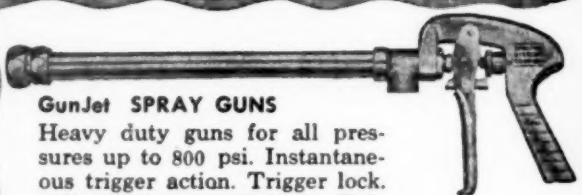

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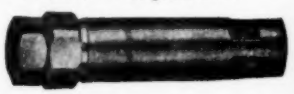

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THE WORLD'S LEADING DESIGNERS AND PRODUCERS OF AGRICULTURAL SPRAY NOZZLES

A BIOLOGICAL ASSAY OF NONVOLATILE, DIFFUSIBLE FUNGICIDES IN SOIL[1]

Donald E. Munnecke

SUMMARY

Plugs of soil treated with a nonvolatile, diffusible fungicide are placed upon potato-dextrose agar media seeded previously with spores of *Myrothecium verrucaria* (Alb. & Schw.) Ditm. ex Fr. After 48 hours, the clear zones surrounding the soil plugs are measured; these provide a quantitative index of the concentration of the fungicides. The technique is reproducible and dependable, and the standard error of individual measurements varies with the concentration and type of fungicides used. The chief advantages of the technique over other means of assay are the speed and ease with which it may be used and the fact that it is not necessary to extract the fungicides. Also, any fungicide may be assayed without alteration of the method, provided its active ingredient will diffuse from the soil and the compound is capable of inhibiting the test fungus.

Although many types of fungicides are used as drenches around living plants, very little is known as to the fate of the fungicides after they are applied to the soil. One reason for the lack of information is that it is difficult to detect the fungicides after they are applied to the soil. The technique described here was developed to aid in studying these nonvolatile, diffusible fungicides; it has been useful in determining the role of soil, concentration of fungicides, soil organisms, and other factors on the effectiveness of nonvolatile fungicidal soil drenches. The technique has been reported previously in abstract form (11).

A number of variations of the antibiotic paper-disc bioassay technique have been developed for measuring fungicide activity. The most precise depend upon assays of aqueous or ethanol solutions of the fungicide absorbed on filter paper discs and applied to the agar (6, 13). The method has been extended to the assay of fungicides on seeds (1, 8) and on leaves (2, 5, 7); the treated seeds or discs of treated leaves are placed directly on the assay medium and the resulting inhibition zones measured. Richardson (12) studied thiram activity in soil by extracting the fungicide from soil with ethanol and assaying the extract by the paper-disc bioassay technique. His method has the limitation that the fungicides must be chemically extracted from the soil. With the technique described here, plugs of soil treated with fungicides are assayed directly. Rapid, reproducible results have been obtained with a number of fungicides.

THE TECHNIQUE.—Plugs of soil treated with a fungicide are placed on potato-dextrose agar seeded previously with spores of *Myrothecium verrucaria* (Alb. & Schw.) Ditm. ex Fr., and are held at 70°–75°F for 48 hours. The diffusion of the fungicide into the agar and the subsequent inhibition of growth of the test fungus result in a clear zone around the soil plug. The diameter of the clear areas is measured in millimeters after 48 hours and used as an index of fungicidal effect.

Thirty-nine g of potato-dextrose agar (Difco Laboratories, Detroit, Michigan) is rehydrated in 1000 ml distilled water, then autoclaved, and cooled to approximately 120°F. Freshly collected spores of *M. verrucaria* in sterilized distilled water are added to the medium to make a suspension containing approximately 250 spores per ml; 15 ml of this suspension is poured into each of the necessary number of sterile Petri dishes (90 mm), allowed to harden, and then held at 33°F until used, preferably within 6 hours after seeding.

Spores are collected by flooding 6- to 8-day-old cultures with small quantities of sterile distilled water and rubbing the spores off the colonies with a blunt transfer needle. One or 2 Petri dishes contain enough spores to prepare 3–4 liters of medium. The spores are mixed with approximately 75 ml sterile distilled water and shaken on a shaking machine for 5 minutes. The number of spores is estimated with a haemacytometer by taking the mean of 4 counts of the spores from the fourth drop from a pipette. A sufficient quantity of the spore suspensions is added to the agar to give a final concentration of approximately 250 spores per ml. The importance of standardizing the concentration of spores will be discussed later.

Oven-dried samples of soil in cylinders, flasks, or other containers are saturated with aqueous solutions or suspensions of fungicides. Assay samples are loosely packed with a spatula into rings (13 mm inside diameter, 13 mm height) cut from plastic tubing. The loosely adhering particles are brushed from the outside surface of the ring, and the plug is gently extruded with the aid of a wooden rod onto the surface of the agar plate without compacting the plug and without breaking the agar surface. Four samples are taken for each replicate.

It is possible for 1 operator to prepare the plates and assay 100–150 plugs in 6–8 hours.

THE TEST ORGANISM.—Several organisms were tested as assay organisms and *M. verrucaria* was selected as most suitable. Its inherent antibiosis to contaminating organisms makes it possible to mix the medium and to pour and handle the Petri plates without special aseptic technique; several thousand plates have been prepared without serious contamination. Other advantages of *M. verrucaria* are that spores disperse in water easily without clumping and the spore suspensions are relatively free of hyphal fragments. Also,

[1] Accepted for publication September 7, 1957.

the growth on seeded plates is extremely dense and uniform, and the color of the colonies furnishes good contrast with the soil; this facilitates photographic recording. The fungus is capable of colonizing soil, and this ability may be useful in evaluating fungicides that may not diffuse into agar, although this aspect has not been investigated by the author.

Sources of error.—The greatest single source of error is the variation in the concentration of spores in the medium, as has been reported by others (3, 9, 10, 12) for other organisms. The greater the concentration of spores, the smaller are the diameters of the zones of inhibition. A concentration of approximately 250 spores per ml was chosen, for this number proved most suited for the technique.

The age of the culture from which spores were obtained had little effect on the sensitivity of the assay, but 5- to 8-day-old cultures are used to minimize a possible variation arising from this source.

A given concentration of a fungicide may affect spore germination in a different manner than it does mycelial growth (4). Since an assay may take 5–6 hours to complete, the physiologic age of the spores in the medium from the beginning to the end of an experiment is a potential source of error. This error is obviated by storing the plates at 33°F immediately after pouring and by using them within 6 hours after they are poured. Tests proved that zones formed on plates stored up to 6 hours at 33°F before fungicides were applied were identical to zones formed on freshly poured plates.

Table 1.—*Standard error (SE) of individual measurements of the diameter of zones of inhibition in the soil-plug assay method, with Myrothecium verrucaria used as test organism* [a]

Fungicide [b]	Concentration of fungicide (ppm active ingredients)	Diameter of zone of inhibition [c] Mean (mm)	SE (mm)
Dithane D-14	1000	61.1	3.3
	500	58.7	5.4
	250	53.2	6.2
	125	42.8	6.3
Captan 50-W	1000	23.1	2.3
	500	20.2	1.6
	250	19.7	3.3
	125	18.0	2.8
Semesan	1000	21.6	2.5
	500	17.8	2.9
	250	15.0	1.4
	125	13.4	0.7
Mixture (1:1) of Fermate and PCNB	1000	28.6	1.7
	500	23.3	3.9
	250	17.3	2.7
	125	14.7	2.9

[a] Number of measurements (N) to determine the mean = 12. Approximately 250 spores/ml potato-dextrose agar.

[b] Fungicides used were as follows: Dithane D-14 (19% nabam, disodium ethylenebis[dithiocarbamate]); Captan 50-W (50% captan, N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide); Semesan (30% 2-chloro-4-(hydroxymercuri)phenol); Fermate (76% ferbam, ferric dimethyldithiocarbamate); PCNB (75% pentachloronitrobenzene).

[c] Data from 4 experiments in which freshly prepared solutions or suspensions of fungicides were used to saturate oven-dried soil (50% peat moss, 50% fine sandy loam) and bioassayed within 1½ hours.

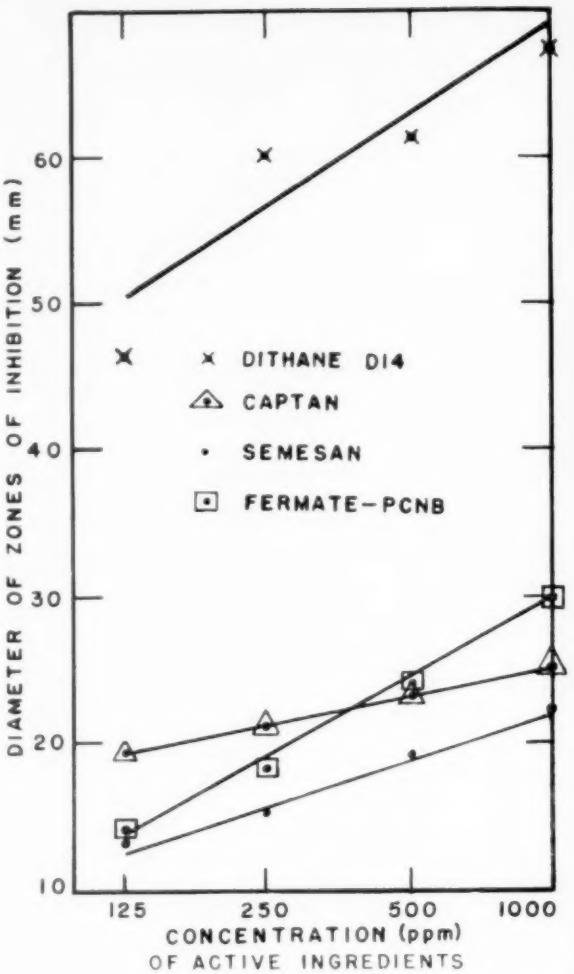

Fig. 1. Dosage-response curves of 4 fungicides assayed by the soil-plug assay technique.

Application of the technique.—The technique has proved to be reproducible and dependable. The standard error of individual measurements varies with the concentration and type of fungicide (Table 1).

Dosage-response curves were plotted from data in Table 1 combined with some from another experiment (Fig. 1). By use of such standard dosage-response curves and the standard error of individual measurements, it is possible to obtain a quantitative index of the concentration of a fungicide in soil. Thus, by plotting the mean diameter of replicate samples on the standard curve, one can determine fungicide concentration. A graded series of doses should be assayed for each experiment, and the curves obtained from these should have slopes and positions similar to those of the

standard curve before the validity of individual measurements of a single assay can be assured. If the curve of the graded series differs radically in slope or position from those of the standard curve, some operation during the experiment was abnormal and the data should be evaluated accordingly.

If the technique is used for qualitative rather than for quantitative purposes, it is not necessary to construct standard curves for each experiment.

DISCUSSION.—The technique has been useful in studies of the factors influencing penetration of fungicides in soil and of the fate of fungicides in soil. It has many advantages over other means of assay, the chief ones being the speed and ease with which it may be used and the fact that it is not necessary to extract the fungicides from soil. Also, any fungicide may be assayed, provided its active ingredient will diffuse from the soil and that the compound is capable of inhibiting the test fungus. Other fungi, including plant pathogens, may be substituted as test organisms; however, care must be taken to standardize the procedure, especially the concentration of the inoculum used in seeding the medium. Eleven fungicides have been studied by means of this technique and all have produced diffusion zones. Since the technique depends upon the abilities of a fungicide to diffuse through agar and to kill or to inhibit the test organism, it cannot be used to compare the fungicidal effectiveness of one fungicide with that of another.

Perhaps the most serious limitation of the technique is its lack of sensitivity at low concentration of fungicides. The exact limit of sensitivity has not been studied, but it is near 100 ppm (active ingredients) of the 4 fungicides reported in Table 1. The sensitivity might be increased by decreasing the concentration of the spores or by using extremely sensitive test organisms.

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BACTERIAL WILT OF BANANAS: DISSEMINATION OF THE PATHOGEN AND CONTROL OF THE DISEASE [1]

Luis Sequeira [2]

SUMMARY

The bacterial wilt of bananas caused by *Pseudomonas solanacearum* E. F. Sm., commonly known as Moko disease, is spread rapidly through banana cultivations by the use of infested tools used in various cultural operations. Infested soil in contact with fresh pruning wounds or other wounds is also an important means of dissemination. The enforcement of a tool-disinfestation program gave almost complete control of the disease in experimental plots.

Infested banana areas can be reclaimed successfully by a combination of disking 5 times during the dry season and fallowing for 9 months before replanting. Apparently, the wilt organism is highly susceptible to desiccation.

The application of bactericidal chemicals such as formaldehyde, D-D, ammonium thiocyanate, hydroxymercurichlorophenol, mercuric bichloride, mercury pentanedione, phenol, potassium permanganate, ferrous sulfate, paraformaldehyde, calcium chloride, or potassium chloride, and of heavy concentrations of fertilizers, such as urea and calcium cyanamid, was ineffective in eliminating the wilt organism from the soil.

The bacterial wilt of bananas caused by *Pseudomonas solanacearum* E. F. Sm., commonly known as Moko disease, is a serious problem to banana growers in many tropical countries. The earliest reference to the disease was made in the 1840's by Schomburgk (15) during his travels in British Guiana. Since then it has been reported from other South American countries, the West Indies, Central America, Southeastern Asia, and Africa. The disease is of particular importance in banana-producing areas of Trinidad, Guadeloupe, Brazil, Costa Rica, Panama, and Honduras. Excellent literature reviews concerning this disease have been prepared by Wardlaw (23) in 1935 and, more recently, by Kelman (7) in 1953.

The etiology and symptomatology of Moko disease are well known from the early work of Rorer (13, 14), Ashby (1, 2, 3), and Wardlaw and McGuire (24). In the initial stages, this bacterial wilt of banana is characterized by the development of a yellowish coloration on the inner leaf lamina at a point close to the petiole. This is followed by wilting of the inner leaves and collapse of the petioles (Fig. 1, A). Progressive wilting of the leaves follows from the center outward. In some cases, no external symptoms are evident until the fruit bunch is produced, at which time individual fruits or "fingers" appear distorted and the pulp exhibits a characteristic dark brown coloration (Fig. 1, B).

Our knowledge of the disease, however, is seriously inadequate in one respect. No observations have been reported on the factors responsible for the rapid dissemination that is characteristic of the bacterium that causes this disease. It has been generally assumed (24) that *Pseudomonas solanacearum* is a normal component of the microflora of certain virgin soils characteristic of low areas with a high water table. The bacterium is able to move into the root system of susceptible solanaceous hosts such as tobacco, tomato and potato through wounds caused by nematodes, soil insects, or other agents(7). Similarly, Wardlaw and McGuire (24) indicated that infection of banana plants was associated with root injury resulting from unfavorable soil conditions.

In the course of extensive investigations carried out by us at Coto on the characteristics and mode of dissemination of the Moko disease organism, it became evident that its entry into banana plants occurs to a large extent through the above-ground plant parts rather than through the roots, unlike the situation just described for other crops. The purpose of this paper is to discuss some of the factors involved in the dissemination of the Moko disease pathogen, the control devised as a result of dissemination studies, and the results of attempts to restore infested areas to banana cultivation through chemical treatments of the soil and through fallowing methods.

A preliminary report of the work included in this paper has been presented (16).

DISSEMINATION STUDIES.—The pseudostem of a banana plant affected by bacterial wilt always shows a large number of discolored vascular bundles usually concentrated toward the inner leaf sheaths and the fruit stalk. Commonly, a number of young suckers growing at the base of a diseased plant also will show symptoms of bacterial wilt (Fig. 1, C) characterized by distortion and splitting of the leaf sheaths. Since it is customary in banana culture to cut off supernumerary suckers at monthly intervals, the presence of bacteria in the discolored vascular bundles of young suckers suggested the possibility that the bacteria might be carried on the blades of pruning knives, hence a simple transmission experiment was set up to test this theory. In addition, the possibility of transmission by infested soil carried to open pruning wounds by rain was also investigated.

METHODS.—Experiments on transmission of the bacteria by sucker pruning were carried out in a banana

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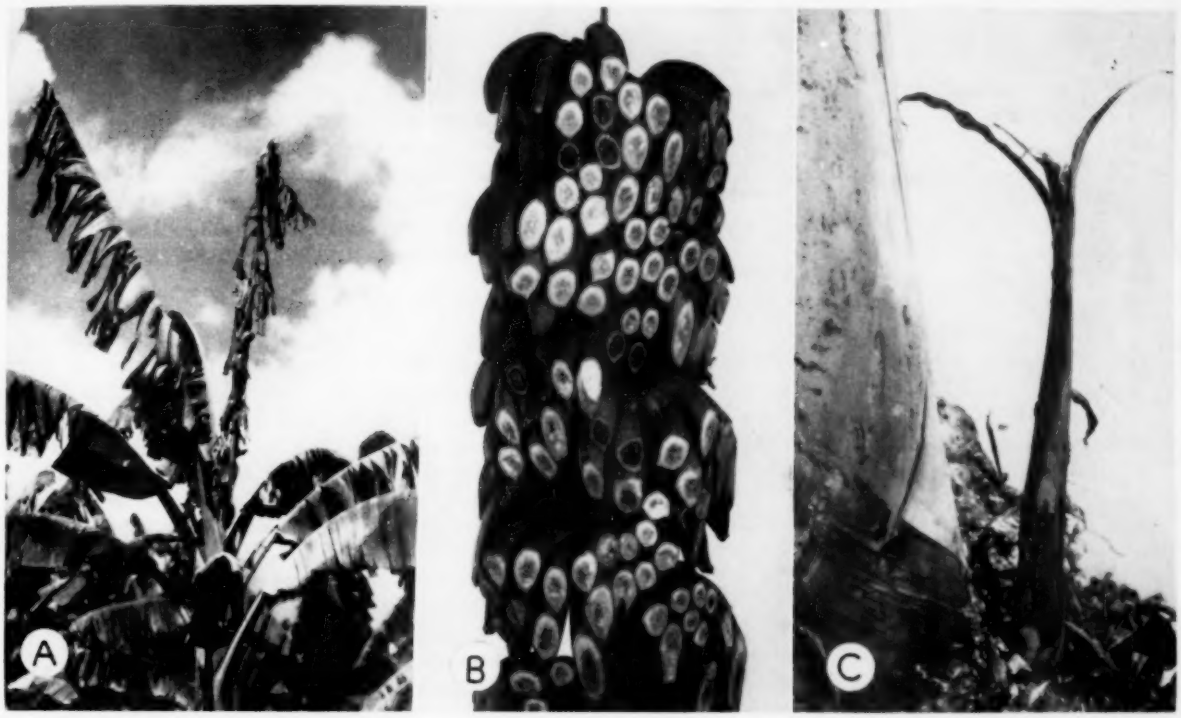

FIG. 1. A) Mature banana plant showing dead inner leaves caused by bacterial wilt. B) Mature banana fruit bunch showing soft pulp rot of some of the individual fruits, a characteristic symptom of bacterial wilt. C) A diseased young banana sucker with split and blackened leaf sheaths. Diseased suckers, such as this shown here, commonly surround the base of banana plants affected by bacterial wilt.

area free of the disease. From 9 to 11 plants, in alternate rows, were treated as follows: 1) suckers were removed with a knife (machete) previously infested by piercing a diseased pseudostem; 2) suckers were removed with an infested machete that was immersed in 5 per cent phenol solution immediately before cutting; and 3) suckers were left intact, to serve as a control.

Ten replications of each treatment were made, so that the entire experiment contained approximately 300 plants. For confirmation, disease surveys were checked by laboratory isolations of the causal organism.

In other experiments designed to test transmission by leaf and flower-bud pruning, the same procedure as above was followed except fewer plants (50–60) were used.

Experiments designed to demonstrate transmission of the bacterial wilt pathogen to above-ground plant parts by means of infested soil were carried out in a wilt-free area. Mature banana plants, which are normally surrounded by a large number of small suckers, were selected for this experiment. Ninety-one plants, arranged in rows of 9–10 plants, were pruned by removing all suckers with a sterilized machete. Alternate rows of plants were then treated by smearing the pruning wounds with soil from an area heavily infested with the wilt bacterium. Remaining rows were left untreated to serve as controls. Disease surveys were made at weekly intervals; for confirmation, laboratory isolations of the causal organism were made from diseased plants.

RESULTS.—Bacterial wilt appeared in nearly all mats pruned with an infested machete, whereas none appeared in those pruned with a disinfested machete (Table 1). Young plants developed disease symptoms earlier than the more mature plants. One case of bacterial wilt appeared in the check group, probably the result of accidental inoculation originating from the large numbers of neighboring diseased plants in the treated groups.

In similar experiments, knives used for pruning of flower buds and leaves were found to transmit the wilt organism. Following bud pruning with a contaminated

TABLE 1.—*Dissemination of Pseudomonas solanacearum by sucker pruning of bananas*

Treatment	No. of plants	Percentage infection After 5 weeks	Percentage infection After 9 weeks
Pruned with infested machete	94	60.5	84.9
Pruned with disinfested [a] machete	95	0.0	0.0
Not pruned	97	0.0	1.0

[a] Dipped in 5 per cent phenol solution.

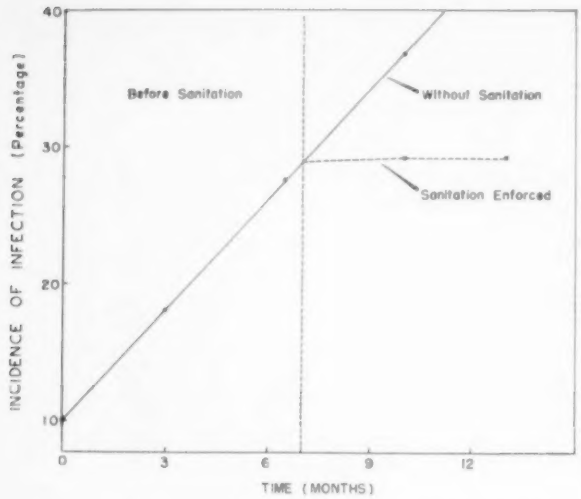

FIG. 2. Rate of spread of bacterial wilt of bananas in areas with and without a sanitation program. Sanitation consisted of tool disinfestation and elimination of all diseased plants.

knife, 100 per cent of the treated plants developed symptoms of bacterial wilt 3 weeks after debudding. Depending on the time elapsed between flower bud infection and harvesting of the fruit, the disease may spread only to individual bananas in the bunch or it may continue downward into the rhizome tissue. Similarly, following leaf pruning with a contaminated knife, all plants pruned developed symptoms of bacterial wilt. When fully matured leaves were pruned, the bacterium moved down the petiole very slowly. In contrast, when a young and actively growing leaf was cut, the organism moved rapidly throughout the plant, all plants showing typical symptoms of bacterial wilt within 7 weeks. In the preceding experiments on leaf and flower-bud pruning, none of the plants pruned with a knife disinfested with 5 per cent phenol solution, or of those left intact as controls, developed wilt symptoms.

In the experiments designed to test the transmission of the wilt organism by infested soil in contact with fresh pruning wounds, equally conclusive results were obtained. Of the suckers treated, 6.6 per cent developed symptoms of bacterial wilt; these diseased suckers belonged to 32.6 per cent of the plants in the treated group (Table 2). It is evident that the wilt organism can spread very rapidly through above-ground parts following direct application of soil from heavily infested areas.

TABLE 2.—*Dissemination of the banana wilt bacterium by infested soil placed on sucker-pruning wounds*

Treatment of wounds	Items treated		Infection after 30 days	
	Suckers	Plants	Suckers	Plants
Smeared with infested soil	No. 339	No. 49	% 6.6	% 32.6
Not treated	373	42	0.0	0.0

CONTROL STUDIES.—*Sanitation procedures to check the spread of bacterial wilt.*—Since pruning operations are carried out approximately every 4 weeks in banana cultivation, it seems probable that most transmission involves above-ground plant parts. In order to determine the importance of this method of transmission, a field test was conducted incorporating the disinfestation of pruning tools and the elimination of diseased plants.

Nine half-acre plots of bananas were selected in areas with an incidence of bacterial wilt varying between light and severe, and the position of all diseased plants was recorded. Regular pruning operations were maintained for the first 7 months, and the rate of disease spread was recorded. After this period, tool disinfestation with 5 per cent formaldehyde solution was initiated, and diseased plants were eliminated with 3 successive applications of a 1 per cent solution of Weedone 32, a mixture of 2,4-D and 2,4,5-T, sprayed on the foliage. A control plot was maintained without these sanitation practices. Records of the incidence of bacterial wilt were maintained for 6 months after sanitation measures were enforced.

Before sanitation was enforced, disease incidence increased by 8-10 per cent of the total plant population between surveys (Fig. 2), and the incidence increased at the same rate in the control plot after sanitation was enforced in the test plots. In contrast, spread was very nearly stopped by the sanitation procedures. In the treated plants, only 1.6 per cent of the plants became infected during the first 3 months after initiation of the program and an additional 0.3 per cent during the next 3 months.

Soil treatments for reclamation of infested areas.—1) *Chemical treatments.*—In the past, many chemicals have been applied to the soil in an attempt to control wilt diseases caused by *P. solanacearum*, such as Granville wilt of tobacco (2,5,12,18) and southern bacterial wilt of potato (10). Although the majority of these chemicals have failed to give adequate control, a few have been considered effective or partially effective.

Since most of the trials with toxic chemicals for the control of *P. solanacearum* were made under temperate-zone conditions, it seemed desirable to test the more promising of these chemicals under tropical-zone conditions. In our experience, replanting of areas abandoned at Coto because of severe infestation by *P. solanacearum* can only be carried out successfully from a commercial standpoint with a period of fallowing of 2 or more years, and under the present conditions it is not feasible to rotate bananas with other crops. Therefore, a method was sought, through the application of chemicals to the soil, that would allow replanting without an extensive fallowing period.

The materials listed in Table 3 under Group I were applied at the sites of infected plants immediately after these plants had been removed; an area of 8 sq. ft. per plant was treated. The plots consisted of rows

TABLE 3.—*Incidence of bacterial wilt of bananas following treatment of infested soil prior to planting*

Expt.	Treatment	Rate	Method of application	Average percentage infection[a]
I	Formaldehyde (18%)	88 ml/sq. ft.	Soil injection	20.0
	Urea	6000 lb./acre	Planting hole	18.5
	D-D	8 ml/sq. ft.	Soil injection	22.0
	None			26.4
II	Mercuric bichloride	25 lb./acre	Drench	36.8
	Potassium permanganate	25 lb./acre	Drench	32.6
	Phenol	50 lb./acre	Drench	34.1
	Hydroxymercurichlorophenol	25 lb./acre	Drench	26.6
	None			27.9
III	Ammonium thiocyanate	1000 lb./acre	Drench	16.1
	Ferrous sulfate	1000 lb./acre	Drench	27.0
	Mercury pentanedione	25 lb./acre	Drench	29.4
	Paraformaldehyde	400 lb./acre	Broadcast	10.1
	None			31.6
IV	Potassium chloride	2000 lb./acre	Broadcast	31.3
	Calcium cyanamid	1000 lb./acre	Broadcast	26.5
	None			31.7
V	Calcium chloride	1000 lb./acre	Broadcast	32.2
	None			44.7

[a] Differences within each group not statistically significant. Duration of experiments I, II, III, IV, and V was 6, 11, 9, 8, and 7 months, respectively.

of 9–11 plants, and alternate plants received the chemical treatments as a single application. Remaining plants were left untreated as a control. Each treatment involved 100–200 plants. Formaldehyde (18% solution) and D-D (1,3-dichloropropene; 1,2-dichloropropane) were injected into the soil with a Mack hand-operated chemical injector[3], 8 ml per sq. ft. Urea was applied by hand at the rate of 20 lb. per plant area (8 sq. ft.) just before planting.

The chemical treatments listed in Table 3 under Groups II, III, IV, and V were carried out in 1/5- to 1/4-acre plots. The plots were cleaned by burning the weeds and removing all debris prior to the chemical applications, but no further preparation of the land was carried out. Three replications of each treatment were made and chemicals were applied either as a drench or were broadcast. Approximately 50 gal. of the solutions used in the drenching experiments were applied to the soil around each individual plant site. Small levees were built around each plot to confine the chemical solutions and to prevent movement between adjoining plots. The chemicals applied by hand were broadcast as evenly as possible but were not mixed into the soil. Replanting to bananas was carried out 2–3 weeks after the treatments were completed.

Bacterial wilt appeared rapidly in all plots, the incidence varying from 10 to 40 per cent, depending on the duration of the experiment (Table 3). An analysis of variance showed that the differences in percentage infection for the various treatments are not statistically significant.

[3] Mack Fluid and Chemical Injector, Model 44AX, manufactured by Mack Anti-Weed Gun, Caldwell, Idaho.

2) *Plowing and fallowing.*—Shallow tillage of infested soil during dry weather conditions has been suggested for control of Granville wilt of tobacco (7). This procedure has been used as a means of reducing the soil moisture to a level detrimental to the pathogen. Small experiments, in which infested soil was disked throughout the dry season, were set up to evaluate this method as a control measure for bacterial wilt of bananas.

During the February-March dry season, four ½- to 1-acre plots, recently abandoned because of bacterial wilt, were disked 5 times to a maximum depth of 10 in. with a disk harrow. Two of these areas were then planted to bananas after a fallowing period of 6 months and 2 were planted after a fallowing period of 9 months. Border strips were planted outside the treated areas as a control. Disease surveys were made at 2-week intervals, and frequent laboratory isolations of the causal organism were made from diseased material obtained from the plots.

The incidence of bacterial wilt was significantly lower in the disked areas than in the control areas

TABLE 4.—*Incidence of bacterial wilt of bananas following replanting of areas treated by disking and fallowing*

Treatments	Fallowing period (months)	Percentage infection after 12 months	LSD(5%)
Disked and fallowed	6	3.9	
Fallowed only	6	18.1	3.4
Disked and fallowed	9	0.7	
Fallowed only	9	6.5	2.8

(Table 4). Additional data not included in Table 4 indicate that when areas abandoned because of bacterial wilt are replanted, without either disking or the combination of disking and fallowing, the disease reappears in 40 per cent of the total population within a period of 6 months. Although fallowing alone results in some control of disease, this procedure is not as successful as when fallowing is combined with disking.

Discussion.—Experimental evidence indicates that the cultural practices peculiar to banana cultivation may be responsible to a marked degree for the rapid spread that is characteristic of bacterial wilt. The high incidence of bacterial wilt in bananas due to the use of infested tools is in contrast with the situation in the cases of bacterial wilt of tobacco, tomato, and other hosts, where the diseases follow root infection; however, Maire (8) suggested that pruning tomato plants with infested knives might be very important in the spread of bacterial wilt. Further, Nakata and Koba (9), Hunger (6), and other authors have indicated that wilt-inducing bacteria could be introduced on the hands or knives of the workers during tobacco-topping operations. Therefore, it seems probable that above-ground infection is of considerable importance in those crops that not only are affected by *P. solanacearum* but also require pruning throughout the growth of the plant.

The possibility of dissemination of *P. solanacearum* to above-ground parts of the banana plant through use of infested tools was recognized by Rorer (13), who recommended that all implements used for cutting or destroying diseased plants be thoroughly flamed or otherwise disinfested. In experiments reported in this paper, the enforcement of a tool-disinfestation program gave almost complete control of disease spread. The few cases that appeared after all precautions were taken to prevent infection might have been due either to invasion through the roots or to contact of infested soil with fresh pruning wounds. Although invasion through the root system does not appear to be responsible for the very rapid spread of bacterial wilt of banana, we know that the organism can penetrate the rhizome tissue readily through the roots of a seed "bit" planted in heavily infested soil. Perhaps a more important means of dissemination is rain-carried infested soil that may splash on fresh wound surfaces, where the wilt bacterium can easily gain access to the vascular bundles of the banana plant.

Some of the chemicals tested in the present work have been considered by other workers to be highly effective against *P. solanacearum* in other crops. For instance, the application of large amounts of fertilizers such as urea (19) and calcium cyanamid (20), as well as chemicals such as ammonium thiocyanate (10), D-D (18), and calcium chloride (20), reportedly has been effective in the control of *P. solanacearum*. Under tropical conditions and at concentrations higher than those reported to be helpful, these materials have been completely ineffective for the control of the banana wilt organism.

The elimination of *P. solanacearum* from soil by a combination of disking and fallowing is undoubtedly due to the extreme sensitivity of the organism to dry conditions and to the absence of suitable plant hosts. In connection with the first of these points, we have determined, following the method of Cabral as described by Dowson (4), that *P. solanacearum* cannot survive atmosphere exposure for 11 days. This is a remarkably short period of survival compared with that of other non-spore-forming plant-pathogenic bacteria; e.g. *Xanthomonas begoniae* (Takimoto) Dows. can survive drying at 25°C for 75 days (4). It is possible, then, to eliminate the banana wilt bacterium by reducing the soil moisture to levels that are detrimental to the organism. In Sumatra, extensive tillage during dry periods has been recommended as a control for tobacco wilt (11), and others (22, 24) have suggested that banana wilt can be controlled by the thorough drying of the soil during the dry season.

In connection with the second point, the absence of suitable plant hosts, we have found that at Coto no infection of tomato, potato, eggplant, and a wide variety of common solanaceous weeds resulted either from direct root or stem inoculation with the banana strain of *P. solanacearum*, even though this organism has been reported to attack a wide range of tropical plants (7). Our results are thus in full agreement with the early work of Rorer (14) and Smith (17), who noted the distinct host limitations of the banana strain of *P. solanacearum*. In spite of these indications, however, it still seems logical to infer that this bacterium must be able to attack some unknown natural hosts that are present in areas not previously planted to bananas, and we are continuing our search at Coto for such possible native reservoirs.

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EVIDENCE OF LOCAL ANTAGONISM BETWEEN TWO CEREAL RUST FUNGI [1]

C. O. Johnston and M. D. Huffman [2]

SUMMARY

Preliminary tests conducted in the greenhouse indicated marked effects of local antagonism between the crown rust of oats organism (*Puccinia coronata* Cda. var. *avenae* Fraser et Led.) and the leaf rust of wheat organism (*Puccinia recondita* Rob. ex Desm., formerly *P. rubigo-vera* (DC.) Wint. f. sp. *tritici* (Eriks.) Carleton). On leaf-rust-susceptible wheat seedlings inoculated with urediospores of the oat crown rust fungus prior to inoculation with the wheat leaf rust fungus, the leaf rust pustules developed were fewer in number and of a different infection type than were those on similar plants inoculated only with the leaf rust organism. Inoculation with the crown rust organism caused a severe chlorotic flecking on the doubly inoculated plants.

Cross protection and acquired immunity are well established phenomena in several virus diseases of plants,[3] but very little evidence has been presented to indicate their operation among other plant pathogens. Yarwood [4, 5] produced the first evidences of cross protection in the fungi by demonstrating antagonism and cross protection between several rust organisms of different genera. The present report presents evidence that a similar mechanism operates between the wheat leaf rust and the oat crown rust organisms.

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[3] Bennett, C. W. 1953. Interactions between viruses and virus strains. Advances in Virus Research 2: 39-67.

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Rust organisms used in these tests were pure cultures of physiologic race 5 of the fungus causing leaf rust of wheat and physiologic race 202 of the fungus causing crown rust of oats. Inoculum was increased on seedling plants of wheat and oat varieties completely susceptible to the races of the leaf rust and crown rust fungi used.

Seedling plants of the leaf-rust-susceptible wheat variety Cheyenne (C.I. 8885) in each of 18 clay pots were inoculated at the second-leaf stage with a uniformly heavy dosage of urediospores of the crown rust fungus. There were 12 to 15 seedlings per pot. Only primary leaves were inoculated. After incubation in a moist chamber for 24 hours, the pots of seedlings were placed on the greenhouse bench at a temperature of approximately 68° F. Seedlings in an equal number of pots were not inoculated but otherwise were treated similarly in all respects. Six pots of seedlings inoculated with the crown rust fungus and 6 pots of control plants were inoculated with urediospores of the wheat leaf rust organism 2, 3, and 5 days following the inoculation with *P. coronata* var. *avenae*. Care was exercised to maintain approximate uniformity in the amount of inoculum used in each case. After an incubation pe-

riod of 10 days following inoculation with *P. recondita*, determinations of the number of pustules per leaf, the pustule type, and the degree of chlorosis were taken on primary leaves of 5 randomly selected seedling plants in each of the 6 pots of the doubly and singly inoculated groups.

Heavy flecking developed on all plants inoculated with the crown rust organism, but no uredia of that fungus developed. The small yellow flecks produced by the crown rust fungus were prominent on all leaves inoculated with that organism, even on plants inoculated with the leaf rust organism only 2 days after inoculation with the crown rust fungus. Considerable chlorosis and tip burning of leaves, caused by the heavy infection by the crown rust pathogen, developed on the doubly inoculated plants that were not inoculated with the leaf rust fungus until 5 days after inoculation with the crown rust pathogen. This was not prominent in groups inoculated with the leaf rust fungus 2 and 3 days after inoculation with the crown rust fungus.

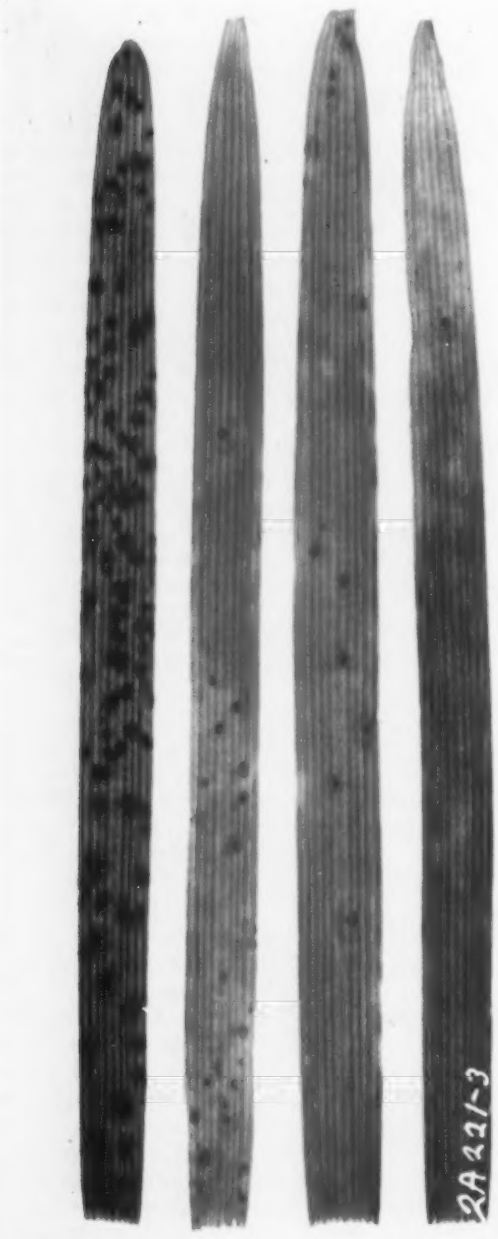

FIG. 1. Response of primary leaves of Cheyenne wheat seedlings to infection by the leaf rust fungus. Left, inoculated only with the leaf rust organism; next 3 leaves to the right inoculated with the crown rust fungus 2, 3, and 5 days respectively, before inoculation with the leaf rust organism.

When plants first inoculated with the crown rust fungus were later inoculated with the leaf rust fungus, there was a marked decrease in the number of leaf rust pustules that subsequently developed (Fig. 1) as compared with the number that developed on control plants not previously inoculated with the crown rust pathogen. The leaf rust pustules that developed on the seedlings previously inoculated with the crown rust fungus were, in general, much smaller than those on singly inoculated plants, although pustule type and size were somewhat variable. Pustules developing in or near chlorotic areas produced by crown rust were very small type-1 infections, whereas those developing at some distance from chlorotic areas were type-4 infections. Secondary leaves that developed after the inoculations with the crown rust fungus were made showed only large, normal type-4 pustules that resulted from inoculation with the leaf rust pathogen. Control plants, inoculated with the leaf rust fungus only, showed no variation in reaction; in all cases they exhibited only susceptible type-4 pustules.

The mild infection type and the small number of pustules of wheat leaf rust developing on leaf-rust-susceptible wheat after prior inoculation with the oat crown rust fungus are interpreted as evidence of antagonism between these species of rust fungi. Inoculation with the crown rust organism inhibits in some way the formation of pustules of leaf rust in the otherwise susceptible tissues; however, this reaction was only localized in these experiments and therefore not analogous to systemic immunity involving the antigen-antibody phenomena developed in animals. The local antagonism indicated in the present report may be one of contact inhibition by the action of substances produced by the latent mycelium of an organism that was not able to establish itself parasitically. In addition, or alternatively, it may result from a reduction in the number of possible infection courts caused by killing or plugging of many of the stomata.

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THE DOWNWARD TRANSLOCATION OF ANTIBIOTICS IN PLANTS[1]

Reed A. Gray

SUMMARY

The antibiotics streptothricin and pleocidin, when sprayed on intermediate leaves of bean or tobacco plants at a concentration of 1 per cent, were readily translocated, in high amounts, downward to older leaves and upward to the young leaves. When the primary leaves of bean plants were sprayed with streptothricin or pleocidin, the young untreated trifoliate leaves did not develop symptoms of common bacterial blight when inoculated with *Xanthomonas phaseoli*. Under the same conditions, streptomycin, dihydrostreptomycin, neomycin, oxamycin, bacitracin, and actinomycin failed to move out of the sprayed leaves in detectable amounts. When primary leaves of bean plants were immersed in a solution containing 1000 ppm streptothricin, the antibiotic was translocated downward to the roots and upward to younger leaves. Several of the antibiotics that failed to move out of sprayed leaves were translocated upward and downward when applied by immersion of 1 leaf in a solution of the antibiotic. Streptothricin and pleocidin, when applied by immersion of 1 leaf in solutions containing 2000 ppm of the antibiotics, were translocated downward through steam-killed sections of petioles and stems to the older leaves below the ring, indicating that downward movement of these antibiotics was principally in the xylem. Streptothricin was also absorbed by the roots and intact stems of plants and translocated upward to all parts of the plant. Streptomycin amine and streptomycin oxime were converted in plants to compounds possessing much higher antibacterial activity than do the original materials.

There have been a number of reports on absorption of antibiotics by roots and stems and upward translocation of such materials in plants, but very little work has been reported on absorption by leaves and downward movement of antibiotics. In a review of the work on translocation of antibiotics in plants, Crowdy and Pramer (5) concluded that penicillin, griseofulvin, and chloramphenicol were absorbed by the roots and translocated to the stems and leaves in all plants tested. Chlortetracycline, streptomycin, oxytetracycline, and mycetin were absorbed by roots and translocated to the stems and leaves of most plants, but there were exceptions in which these materials failed to move in 1 or 2 plant species. Gramicidin, neomycin, pyocyanin, and subtilin apparently were not translocated from the roots to the stems and leaves.

Spraying the foliage of plants with antibiotics for controlling plant diseases seems to be a more practical method of application than treating the soil in which the roots are growing. Therefore, in the work reported here, the leaves of plants were treated with antibiotics, and the transport of the antibiotics out of the leaves and up the stem to the young leaves or down the stem to the old leaves and roots was traced. Such a study should aid in finding antibiotics that might be useful for controlling vascular and root diseases that are not affected by the conventional surface protectants. It should also add to our knowledge of the particular chemical structures required for downward translocation. The use of antibiotics facilitates translocation studies in plants, because small amounts can be easily detected in the various plant parts by bioassay methods provided no antibacterial agent occurs naturally in the plant.

GENERAL METHODS AND MATERIALS.—Plants were grown individually in 3-in. pots of soil in the greenhouse at 26° C. The soil was kept moist at all times. When certain leaves of a plant were sprayed with an antibiotic, the remainder of the plant was shielded by wrapping it tightly in aluminum foil or by enclosing the plant within a cardboard cylinder with the leaf to be sprayed sticking out through a slit in the cardboard. The sprays were applied to the upper and lower surfaces of the leaves until small droplets formed but did not run off. The spraying of the primary leaves of a bean plant with a 1 per cent solution of an antibiotic resulted in the deposition of about 0.75 ml of solution, or 7.5 mg of antibiotic on each leaf. To determine the amount of antibiotic translocated, the untreated parts of the plant were frozen and thawed; the juice was pressed out and assayed by the paper-disc method on agar plates seeded with *Bacillus subtilis* Cohn emend. Prazmolski or with *Micrococcus pyogenes* var. *aureus* (Rosenbach) Zopf. The antibiotic standards were prepared in juice pressed from untreated bean leaves. Smaller inhibition zones were produced when bean leaf juice was used than when water or buffer was used for the antibiotic standards. Zone sizes were about the same with tomato and tobacco leaf juices as they were in bean leaf juice. The sensitivity of the assays varied slightly from 1 day to another.

All antibiotics were of the highest purity obtainable. Streptomycin, dihydrostreptomycin, penicillin, oxytetracycline, chloramphenicol, chlortetracycline, neomycin, bacitracin, oxamycin, and viomycin were the purified salts used for medical purposes. The streptothricin used was quite pure, having an activity of 500 units/mg based on the streptomycin assay. Pleocidin had approximately the same purity as the streptothricin, and actinomycin had about the same activity as pure streptomycin. The xanthomycin sample was a crude preparation.

In some cases, antibiotics were applied by immersion

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of 1 leaf of a plant in a solution of the antibiotic contained in a glass vial. The leaf was gently rolled into a cylinder to get it into the vial, then 40 ml of the antibiotic solution was added with a pipette. The vial was supported on a bamboo stake by means of Scotch tape and covered with a piece of heavy cheesecloth to prevent heating of the solution.

RESULTS.—*Translocation of antibiotics from sprayed primary bean leaves to the first trifoliate leaves.*—In the initial experiments, the primary leaves of Pinto bean plants at the age when their first trifoliate leaves were about half expanded were sprayed with an aqueous solution containing 1 per cent of the antibiotic while the remainder of the plant was shielded from the spray. Another spray was applied the following day, and the untreated first trifoliate leaves were sprinkled with Carborundum and rubbed with a suspension of the common bean blight bacteria, *Xanthomonas phaseoli* (E. F. Sm.) Dows. Six days after inoculation, the common blight lesions were counted on the young trifoliate leaves. These leaves were then harvested and assayed for antibiotic content. Three plants were used for each antibiotic treatment, and each plant was assayed individually.

No translocation to the young leaves was detected with streptomycin, dihydrostreptomycin, neomycin, oxamycin, bacitracin, xanthomycin, and actinomycin. The antibiotics streptothricin and pleocidin were readily absorbed by the leaves and were transported out of the primary leaves and up the stem to the young trifoliate leaves (Table 1). Transport to the young trifoliate leaves was evident both by bioassay and by protection from common blight symptoms. Streptothricin and pleocidin both caused injury to the treated leaves, as evidenced by the appearance of necrotic spots.

Upward translocation of streptothricin.—For a better understanding of the downward translocation of streptothricin in plants, a knowledge of its translocation upward when applied to the roots was desired. Three Pinto bean plants growing in sand were watered twice with Hoagland's nutrient solution (as modified by Walker and Hooker (8)) containing 2500 ppm streptothricin; the second watering followed the first by 2 days. Four days after the first treatment, the leaves, stems, and roots were harvested, and the roots were washed 10 minutes in running water. Each part was assayed as described above. Streptothricin was absorbed by the roots and translocated upward to all parts of the plant (Fig. 1.A).

In another experiment, streptothricin was absorbed by the roots of tomato plants growing in nutrient solution containing 200 ppm of the antibiotic and translocated to the leaves, where it was found at concentrations of 86 ppm in the juice of older leaves (third and fourth above cotyledons) and 150 ppm in the 2 youngest leaves (seventh and eighth above cotyledons). In another plant, the concentration was 115 ppm in the juice of the intermediate leaves (fifth and sixth above cotyledons).

A study also was made of the absorption of streptothricin by stems and its translocation upward. Pinto bean plants growing in soil were sprayed on that portion of the stem below the primary leaves with solutions containing 0.25, 0.5, or 1 per cent of streptothricin. The sprays were applied until small droplets formed and adhered to the stem. The rest of the plant was shielded from the spray by enclosing it tightly in wrapping paper. The stems were sprayed again the following day, and in some cases the spray was repeated twice on the third day. The first trifoliate leaves were inoculated with common bean blight bacteria on the day following the final spray as described previously. Six days later, the lesions were counted and the first trifoliate leaves were harvested and assayed for streptothricin as described above.

Streptothricin was absorbed by the intact stems and translocated several inches up the stem to the first trifoliate leaves, where it was found in high amounts and where it protected the leaves from the common bacterial blight of beans (Table 2). No injury resulted from such treatments, except for slight browning on small areas of the stem.

Translocation of streptothricin and pleocidin upward and downward from sprayed intermediate leaves of bean and tobacco plants.—The distribution of streptothricin through bean plants following the spraying of certain intermediate leaves with streptothricin was determined. A spray containing 1 per cent streptothricin was applied on 2 successive days. The petiole of the leaf to be sprayed was wrapped with filter paper to prevent any drops of streptothricin from running down to the stem, and the rest of the plant was tightly shielded from the spray. The untreated leaves, stems, and roots were harvested 5 days after the first spray and assayed as described above.

When 1 primary leaf was sprayed, translocation to the opposite primary leaf and up to the young leaves

TABLE 1.—*Common blight lesions on, and antibiotic content of, inoculated trifoliate leaves of bean plants when primary leaves were sprayed with various antibiotics*

Treatment applied to primary leaves[a]		Data obtained with first trifoliate leaves	
Antibiotic	Spray concentration	No. of lesions[b]	Antibiotic content[c]
	%		ppm
None		216, 256	—, —
Streptothricin	0.25	65, 59	—, —
	0.50	1, [e]	—, 200
	1.0	0, [e]	180, 266
	1.0[d]	[e], [e]	300, 800
Pleocidin	1.0	0, [e]	45, 41

[a] Sprays applied on first and second days.
[b] Average number of lesions per leaf for 3 leaves counted 6 days after inoculation on the second day.
[c] Leaves harvested on sixth day after inoculation except as noted; a minus sign indicates that the antibiotic was not detectable, i.e. less than 6 ppm.
[d] Leaves harvested 3 days after spraying rather than 6.
[e] Not run.

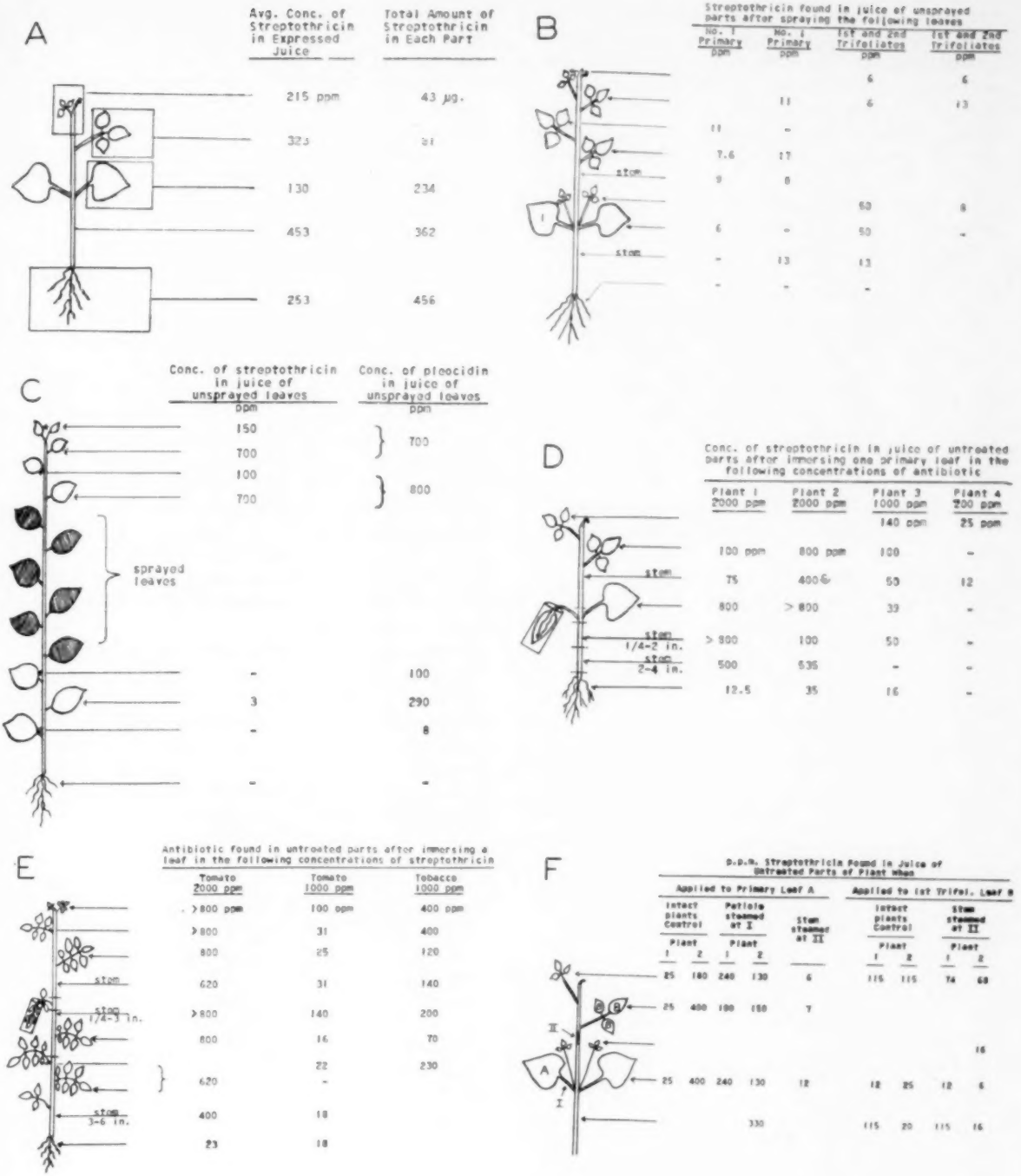

FIG. 1. Antibiotic content of different parts of plants following application of streptothricin or of pleocidin to various plant parts by different methods. Plants were harvested 3–5 days after treatment. A) Bean plants growing in sand watered twice with nutrient solution containing 2500 ppm streptothricin. Figures are averages for 3 plants. B) Bean plants, where a single primary leaf or first and second trifoliate leaves were sprayed on 2 successive days with 1 per cent streptothricin. C) Tobacco plants, following spraying of intermediate leaves with solution containing 1 per cent glycerol and 1 per cent streptothricin or 1 per cent pleocidin. D) Bean plants, following immersion of a primary leaf in streptothricin solutions. Plants 1 and 2 and plants 3 and 4 were assayed 2 and 4 days, respectively, after treatment was started. E) Tomato and tobacco plants, following immersion of an intermediate leaf in streptothricin solutions for 5 days. F) Bean plants, with steam-killed sections at I and II, following immersion of primary leaf or first trifoliate leaf in 1000-ppm streptothricin for 3 days.

was detected (Fig. 1,B). When the first and second trifoliate leaves were sprayed, streptothricin was translocated down the stem to the old primary leaves and to the young trifoliate leaves attached at the primary leaf axils. It also was translocated up the stem to the very young leaves. This experiment clearly shows that streptothricin is translocated downward from sprayed leaves in bean plants.

The 6 intermediate leaves of White Burley tobacco plants 1 ft. tall were sprayed once with 1 per cent streptothricin or 1 per cent pleocidin solution; the other leaves and stems were shielded with aluminum foil. Both sprays contained 1 per cent glycerol to increase absorption (6). The untreated leaves were harvested 4 days after spraying and assayed as described above. Pleocidin was translocated in high amounts down to the older leaves and up to the young leaves. Streptothricin was found in only 1 of the older leaves below the treated leaves, but it was found in high amounts in young leaves above the sprayed leaves (Fig. 1,C). This experiment demonstrates that streptothricin and pleocidin can be translocated upward and downward from sprayed leaves in tobacco plants.

Transport of streptothricin from leaves immersed in solutions of the antibiotic.—Because of the great care that had to be taken in shielding the untreated leaves from the spray in previous experiments, a simpler method was investigated. One leaf of a bean, tomato, or tobacco plant was immersed in a solution of the antibiotic contained in a vial. The leaves remained immersed in the antibiotic solution until the untreated plant parts were harvested 2 or 5 days after treatment and assayed.

The antibiotic was distributed throughout all parts of a bean plant after the immersion of 1 primary leaf in a solution of streptothricin (Fig. 1,D). When the vial contained 2000 ppm. streptothricin was translocated in small amounts down to the roots; it was translocated in high amounts to the stem below the treated leaf, to the stem and leaves above the treated leaf, and to the opposite primary leaf. When 1000 ppm streptothricin was applied, considerable amounts were translocated and no toxicity was noticed. Very little translocation took place when the leaf was immersed in 200 ppm of the antibiotic, but some movement was detected.

When intermediate leaves of Rutgers tomato plants or of White Burley tobacco plants were immersed in a solution of streptothricin, the antibiotic moved in high amounts to the leaves above and below the treated leaf (Fig. 1,E). Some antibiotic activity was detected in the tomato roots, but this probably was not due to streptothricin, since the roots of untreated tomato plants contained an antibacterial agent equivalent to 10-15 ppm of streptothricin. The other parts of the tomato plant did not contain this material.

In a short-time experiment of 4 hours, pleocidin moved out of the treated leaf of a 3-ft. tobacco plant and 6 in. up the stem to the second leaf above the treated leaf, where it was detected at a concentration of 17 ppm. The first leaf above the treated leaf contained 9 ppm pleocidin and the third leaf below the treated leaf contained 17 ppm 4 hours after treatment started. None of the other 8 leaves above the treated leaf or the other 5 leaves below the treated leaf contained detectable amounts of the antibiotic. In other tobacco plants treated in the same way, no movement to other leaves could be detected in shorter time periods of 2 hours or 1 hour.

Systemic action of streptothricin against common bean blight.—In the previous experiment, in which a primary leaf of a bean plant was immersed in a solution containing 1000 ppm of streptothricin, transport throughout the plant occurred in high amounts, yet the antibiotic was not toxic to the untreated parts. Therefore, streptothricin was tested against the common bacterial blight of beans under the same conditions; four plants were used for each treatment. When the antibiotic was applied to the primary leaf at the time the first trifoliate leaves were inoculated, complete protection was obtained as shown by the absence of lesions on 12 leaflets 7 days later. When the antibiotic was applied 2 days after inoculation, 1210 lesions developed on the 12 untreated trifoliate leaflets whereas 4630 lesions developed on 12 trifoliate leaves of untreated control plants.

Testing the translocation of a number of antibiotics by the leaf-immersion method.—The translocation of a number of antibiotics was tested by immersing an intermediate leaf on a tobacco or bean plant in a vial containing 2000 ppm of the antibiotic in aqueous solution. All antibiotics were tested at the same time under one set of conditions. After 5 days of immersion, the young leaves and the portion of the stem

TABLE 2.—*Streptothricin content of unsprayed inoculated trifoliate leaves and the number of common blight lesions on such leaves following the application of streptothricin to stems of bean plants*

No. of sprays[a]	Interval between last spray and assay (days)	Concentration of spray %	No. lesions per leaf[b]	Streptothricin concentration ppm
None			>500	
2	7	1	6	400
			0	500
2	3	1		300
				400
None			236	
4	7	0.25	1	100
			0	100
		0.50	0	150
			0	150
		1.0	0	200
			0	300

[a] Sprays applied to stem below primary leaves on 2 or 3 successive days, with 2 sprays on the third day where applied.

[b] Trifoliate leaves inoculated with *Xanthomonas phaseoli* on day following final spray.

above the treated leaf, as well as the old leaves, stem, and roots below the treated leaf, were harvested. Juice was expressed from each part and assayed for antibiotic content. Under these conditions, higher amounts of the antibiotics were absorbed by the leaves than when application was by spraying; as a result, translocation could be detected with antibiotics that apparently were not translocated following application by spraying.

Following application by the leaf-immersion method evidence of transport out of the treated leaf and up the stem to the younger leaves in tobacco plants was obtained with the following antibiotics: penicillin, streptomycin, neomycin, streptothricin, pleocidin, and viomycin (Table 3). Chlortetracycline, oxytetracycline, chloramphenicol, oxamycin, bacitracin, and actinomycin did not move out of the treated leaf and up to the young leaves of tobacco in detectable amounts in this experiment. The only antibiotics that were translocated to the older leaves below the treated leaf were streptothricin, pleocidin, and neomycin. Neomycin was found at a very low concentration in such tissues.

In Pinto bean plants (Table 3), movement from the treated primary leaf to the young trifoliate leaves occurred with chloramphenicol, penicillin, streptomycin, streptothricin, pleocidin, and viomycin but did not occur with chlortetracycline, oxytetracycline, bacitracin, and oxamycin. The same antibiotics that moved to the young leaves, with the exception of chloramphenicol, also moved to the opposite primary leaf. Neomycin also was found in the opposite primary leaf.

In all tests on tobacco plants, with the exception of those with penicillin, the antibiotics that were translocated to the upper leaves also were found in the stem above and below the treated leaf. Besides these, chlortetracycline and oxamycin were found in the stem just below the treated leaf. In bean plants, chlortetracycline, chloramphenicol, penicillin, streptomycin, neomycin, streptothricin, and pleocidin were found in the stem above and below the treated leaves whereas oxytetracycline, bacitracin, oxamycin, and viomycin were not detected in the stem above or below the treated leaf.

These results must be considered preliminary, since 1 tobacco plant and 1 bean plant were used for each antibiotic. Some of the antibiotics that failed to move in this experiment were translocated in detectable amounts when applied to different leaves on bean plants, when tested under different environmental conditions, or when tested on tomato. In additional tests, the first trifoliate leaf instead of the primary leaf of bean plants was immersed in the antibiotic solution so that movement to the primary leaves could be observed. Translocation of the antibiotics in Rutgers tomato plants also was studied. The sixth leaf from the base of tomato plants that were 1 ft. tall and that had 10 leaves was immersed in a solution containing 2000 ppm of the antibiotic. After immersion for 3 days, the untreated leaves and stems were harvested and assayed as described above.

In bean plants, all the antibiotics tested were translocated to some extent from the first trifoliate leaf to the young trifoliate leaves and also to the old primary leaves (Table 4). Chlortetracycline was found in very minute amounts. Stability studies showed that this was not due to destruction of the antibiotic in the vial. Penicillin also was transported to the primary leaves in very small amounts. Large amounts of viomycin and of streptothricin moved to the old primary leaves. In the youngest leaves, the concentration of streptomycin was higher than that of the other antibiotics. Neomycin moved to the lower portion of the stem in a higher concentration than did any of the others.

In tomato plants, all the antibiotics tested by the leaf-immersion method were translocated from an intermediate leaf up to some of the younger leaves and also down to some of the older leaves (Table 4); however, chlortetracycline was found in concentrations of less than 1 ppm. Streptothricin reached higher concentrations in the youngest leaves and in the oldest leaf than did any other antibiotic. It also was found in the lower portion of the stem in high concentrations. These results indicate that most antibiotics can move up and down from intermediate bean and tomato leaves immersed in solutions of antibiotics. Translocation of most of the antibiotics to other parts of bean plants was greater when the antibiotics were applied to the first trifoliate leaves in this experiment than when applied to the older primary leaves in the previous experiment. Changes in conditions, such as the amount of light intensity, could have been responsible for this difference.

All the antibiotics tested except penicillin caused some toxicity to untreated parts of bean plants. Toxicity was less in tomatoes than in beans; when present, it usually appeared as chlorosis in the young untreated leaves. Chloramphenicol was the only one that produced necrotic areas on the untreated tomato leaves.

In 1 experiment, various forms of streptomycin were compared to see if some were translocated better than others in bean and tomato plants. Streptomycin sulfate, dihydrostreptomycin sulfate, mannosidostreptomycin sulfate, streptomycin desoxycholate complex, streptomycin amine, and streptomycine oxime were applied by the leaf-immersion method, each at a concentration of 2000 ppm. Assays, which were run on the untreated leaves 3 days after application, showed that streptomycin sulfate moved upward and downward in both plants in slightly higher amounts than did the dihydro or mannosido derivatives. The amine and oxime seemed to move downward and become better distributed in the tomato leaves and stem below the treated leaf than did streptomycin sulfate.

Both streptomycin amine and the oxime apparently were converted in the plants to materials with much higher antibiotic activity than that possessed by the amine and the oxime. A standard solution of streptomycin amine in the bean leaf juice at a concentration of 2000 ppm gave an inhibition zone of 23 mm with

TABLE 3.—*Antibiotic content of different plant parts following a 5-day immersion of single intermediate leaves of tobacco or single primary leaves of beans in solutions containing 2000 ppm of various antibiotics*

Antibiotic	Least amount detected in juice (ppm)	Ppm in tobacco plants[a]								Ppm in bean plants[a]				
		Above treated leaf				Below treated leaf				Above treated leaf			Opposite primary leaf	Stem below treated leaf
		Leaves 1–2	Leaves 3–4	Leaves 5–7	Stem	Leaves 1–2	Leaves 3–4	Stem	Roots	Leaf 1	Leaves 2–3	Stem		
Chlortetracycline	3	—	—	—	—	—	—	16		—	—	6	—	3
Oxytetracycline	12	—	—	—	—	—	—	—		—	—	—	—	—
Chloramphenicol	6	—	6	—	—	—	—	—		16	35	9	—	8
Penicillin	<1	—	3	1	—	—	—	—		1	25	5	50	3
Streptomycin	<1.5	15	80	100	50	—	—	35	—	9	15	11	15	11
Neomycin	<1.5	—	6	12	12	—	2	25		—	—	15	18	150
Bacitracin	6	—	—	—	—	—	—	—		—	—	—	—	—
Actinomycin	<1.5	—	—	—	—	—	—	—	—					
Oxamycin	25	—	—	—	—	—	—	54		—	—	—	—	—
Streptothricin	3	6	230	15	60	10	—	43	—	13	50	12	76	10
Pleocidin	3	9	400	43	43	160	700	30	—	30	9	30	36	12
Viomycin	12	—	16	310	50	—	—	200		70	70	—	250	—

[a] Leaves numbered from treated one. Each figure is for a single plant. Minus sign indicates that antibiotic was either absent or present in amounts too small for detection.

TABLE 4.—*Antibiotic content of different plant parts following a 3-day immersion of single intermediate leaves of tomato or of single first trifoliate leaves of beans in solutions containing 2000 ppm of various antibiotics*

Antibiotic	Ppm in tomato plants[a]										Ppm in bean plants[a]						
	Above treated leaf					Below treated leaf					Above treated leaf			Below treated leaf			
	Leaf 1	Leaf 2	Leaf 3	Leaves 4–5[c]	Stem[b] ¼–3 in.	Leaf 1	Leaf 2	Leaf 3–4	Stem[b] ¼–3 in.	Stem[b] 3–6 in.	Leaf 1	Leaf 2–3[c]	Stem[b] ¼–8 in.	Primary A	Primary B	Stem[b] ¼–6 in.	Stem[b] 6–12 in.
Penicillin	12	200	280	100(—)	6	10	35	12	8	3	400	125(—)	50	<1	<1	8	<1
Chlortetracycline	—	<1	<1	<1(C)	<1	—	<1	<1	2	<1	2	<1(N)	2	<1	<1	12	2
Oxytetracycline	6	20	35	10(C)	16	6	5	5	64	12	35	20(N)	5	35	50	50	8
Chloramphenicol	5	40	280	50(N)	25	—	32	16	32	22	>400	200(N)	400	64	44	400	13
Neomycin	—	25	50	1(—)	25	—	5	4	25	12	>400	—(W)	400	400	50	6	100
Streptomycin	10	78	200	42(C)	30	50	2	30	36	8	>400	400(C)	400	200	50	80	—
Dihydrostreptomycin	200	>400	>400	150(C)	250	100	20	42	100	30	>400	60(C)	100	280	200	50	1
Viomycin	84	240	240	10(—)	330	—	66	—	>400	140	>400	10(N)	10	>400	>400	280	—
Streptothricin	64	>400	>400	400(—)	400	15	42	56	>400	200	>400	35(N)	200	>400	>400	>400	15
Pleocidin	6	18	50	—(—)	60	—	6	—	170	84	>400	44(N)	140	200	170	320	25

[a] Leaves numbered from treated one. Each figure is for 1 plant. Minus sign indicates that antibiotic was either absent or present in amounts too small for detection.
[b] Stem measured from node of treated leaf.
[c] Letters in parentheses indicate injury to young untreated leaves as follows: — = none, N = necrotic spots, C = chlorosis, W = wilting.

B. subtilis. A solution of 400 ppm gave an inhibition zone of 19 mm with *B. subtilis* and a growth zone of 27 mm with the streptomycin-dependent strain of *Escherichia coli*. A concentration of 200 ppm gave a 15-mm inhibition zone and no growth zone. The juice from the leaf above the treated leaf of a bean plant gave an inhibition zone of 30 mm with *B. subtilis* and a growth zone of 37 mm with *E. coli*. The juice from the leaf below the treated leaf of a tomato plant gave an inhibition zone of 28 mm and a growth zone of 32 mm. Thus, the juice of untreated leaves had higher antibiotic activity than did the applied solution. Streptomycin oxime gave similar results. It appeared that streptomycin amine and the oxime either were converted in the plants to materials with much greater antibiotic activity or were translocated in very high amounts to the point of accumulation. The first explanation seemed the more plausible, since the pure amine and oxime did not produce such large zones even at extremely high levels, and accumulation at even higher levels in the untreated leaves seemed unlikely. The conversion product was probably streptomycin or dihydrostreptomycin, since these are the only known compounds that are active at low levels in the growth test with *E. coli*.

Pathway of movement of streptothricin and pleocidin in plants.—Several experiments were made to determine the conducting tissue involved in the downward movement of streptothricin in plants. Sections of bean stems or petioles of leaves to be treated were killed by steaming, and translocation through these killed stems was compared with that through stems of intact plants. Streptothricin, at a concentration of 1000 ppm, was applied by the leaf-immersion method to a primary leaf of each of several intact plants and ringed plants and to the first trifoliate leaf of others. The untreated leaves and stems above and below the steamed sections were harvested 3 days after treatment started and assayed as described above. Movement was about the same through steam-killed stem sections as it was in intact plants (Fig. 1,F). Since streptothricin moved through killed petioles and downward through steam-killed stem sections in amounts similar to those that moved in intact plants, it is concluded that streptothricin, when applied by immersion of the leaf in the antibiotic solution, moves downward in bean plants principally in the xylem. This is the opposite of what one would expect from the available information on the downward movement of other organic solutes, which move downward mainly in the phloem tissue (4). Streptothricin also moved upward in the main stem through a steam-killed section, an indication that upward movement of streptothricin also occurs in the xylem tissue.

The downward translocation of pleocidin through steam-killed petioles and stems was traced in tomato plants. Pleocidin applied by immersing 1 intermediate leaf in a 1000-ppm solution of the antibiotic moved out of the treated leaf through a steamed ring in the petiole and into the young leaves, where it was found at concentrations of 25 and 70 ppm in the leaf juice. The stem sections above and below the treated leaf contained 38 and 47 ppm pleocidin, respectively. Pleocidin also moved out of a treated leaf, through a steam-killed section of the main stem, and into the old leaves below the ring, where it was found in concentrations of 130, 8, and 2 ppm, and to the stem below the ring where it was found at a concentration of 230 ppm in the expressed juice. Movement in intact plants was slightly greater than it was in the ringed plants, but it was concluded that pleocidin, like streptothricin, is translocated downward principally in the xylem under the conditions used.

In these experiments where streptothricin and pleocidin were shown to move downward through the xylem, the antibiotics were applied by immersing 1 leaf of a plant in a solution of the antibiotic. It is unlikely that downward transport occurs in the xylem when leaves are sprayed with these antibiotics. On testing this principle further, it was found that the plant growth regulators 2,4-dichlorophenoxyacetic acid and gibberellic acid, moved downward through steam-killed sections of petioles when the leaves were immersed in solutions of 100 ppm of the compound in each case. Evidently under certain conditions a reduction in pressure occurs in the xylem so that, when leaves are immersed in an aqueous solution, the solution is drawn into the xylem.

Discussion.—Streptothricin and pleocidin were the only antibiotics tested that moved readily in high amounts out of sprayed leaves and up and down the plant. It would be helpful in predicting the translocation of other antibiotics if the structures of these antibiotics were known. Streptothricin was first isolated by Waksman and Woodruff in 1942 (7). It is produced by an actinomycete, *Streptomyces lavendulae* Waksman & Curtis. The chemical structure of streptothricin has been partially determined by Carter et al (1), who found that it has the empirical formula $C_{20}H_{34}N_8O_9{\cdot}3HX$. Streptothricin gives positive ninhydrin and biuret tests and a negative Sakaguchi test. It has 3 basic groups, no carboxyl groups, and 2 primary amino groups. On hydrolysis, 6 of the 8 nitrogen atoms appear as primary amino groups, but no α-amino acids are formed. Three compounds are produced on hydrolysis; the main constituent was found to be β,ε-diamino-n-caproic acid, which is also called β-lysine.

Pleocidin is a streptothricin-like antibiotic produced by an actinomycete resembling *S. lavendulae*. Its production and purification were described in 1953 by Charney et al (2). Another streptothricin-like antibiotic, Sharp & Dohme #896, also was translocated downward in plants when sprayed on leaves.

When a single primary leaf of a bean plant was immersed in a solution of streptothricin, the young leaves that were inoculated with the bacterial blight organism were protected from the disease. Other antibiotics also became systemic when applied by this method to bean, tomato, and tobacco plants. This leaf-

immersion method of applying streptothricin and other antibiotics might be useful for treating certain valuable ornamental plants for virus, bacterial, and fungus diseases. Similarly, spraying the stems of plants with systemic antibiotics such as streptothricin may find application for controlling diseases of certain plants. Results reported above showed that bean stems were very efficient absorbing-tissues for streptothricin, as shown by the high amounts translocated to the leaves after application as a spray to the lower portion of the stems.

Since application of antibiotics by immersion of a leaf in a solution of the antibiotic results in translocation under conditions where application by spraying of the leaves does not, this method is not suitable for predicting which antibiotics will be translocated when they are sprayed on the leaves. It is felt that translocation occurs following leaf immersion because of the relatively large amounts absorbed by the leaves or because leaf immersion facilitates xylem transport. In 2 bean plants where 1 per cent glycerol was added to increase the absorption of streptomycin sprayed on the primary leaves (6), the juices of the young trifoliate leaves were found to contain 6 and 12 ppm of the antibiotic. Trifoliate leaves of 2 plants similarly sprayed but without glycerol contained no detectable antibiotic.

The apparent ease of movement of the antibiotics streptothricin and pleocidin downward through steam-killed sections of petioles and stems was rather surprising at first, since most organic solutes fail to pass a steamed ring, as indicated in a recent review by Crafts (4). The antibiotics were applied by the leaf-immersion method in the experiments where downward translocation in the xylem was noted. It is felt that they probably would not do the same when applied by spraying the leaves. Since 2,4-dichlorophenoxyacetic acid and gibberellic acid also moved downward through steam-killed petioles of bean plants when the leaf blades of the same leaves were immersed in solutions of the growth regulators, it appears that streptothricin and pleocidin are not unique in this respect. It was not clear in the literature on translocation that organic solutes must be applied as small droplets to leaves and not by leaf immersion in order to get phloem transport and to avoid xylem transport; however, Colwell (3) obtained downward movement of an inorganic material (P^{32}) past a steamed ring in the stem of a squash plant when 10 ml of $Na_2HP^{32}O_4$ solution was applied in a plasticine lake on the leaf. Apparently, downward movement in the xylem occurred only when the plants had a water deficit in the xylem.

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PHYSIOLOGICAL SPECIALIZATION IN COLLETOTRICHUM LAGENARIUM [1]

Monroe J. Goode [2]

SUMMARY

Colletotrichum lagenarium was isolated from severely infected plants of the anthracnose-resistant watermelon varieties Congo and Charleston Gray at 4 locations in eastern North Carolina in 1954 and 1955. Isolates derived from single spores of this fungus were obtained also from various other cucurbits including both anthracnose-resistant and anthracnose-susceptible varieties of watermelon, cucumber, cantaloupe, squash, and pumpkin. Among these isolates, 3 physiological races were distinguished by their ability to attack certain cucurbit seedlings and fruits. The 3 races were indistinguishable culturally and morphologically. Race 1 attacked Butternut squash plants moderately but was virulent on all cucumber varieties tested as well as on anthracnose-susceptible varieties of watermelon. It caused only slight infection on Charleston Gray, Congo, and Fairfax watermelon seedlings, and the inoculated plants subsequently recovered and continued to grow normally. Race 2 was moderately pathogenic on Butternut squash and was highly pathogenic to all watermelon and cucumber varieties tested. Butternut squash was immune from Race 3, which only rarely caused a superficial fleck-type reaction on leaves of Congo, Charleston Gray, and Fairfax watermelon seedlings. This race was highly pathogenic to all of the varieties of cucumber and anthracnose-susceptible watermelon varieties tested. Potential sources of resistance to all 3 races were found in both watermelon and cucumber.

Anthracnose, incited by *Colletotrichum lagenarium* (Pass.) Ell. & Halst., is a destructive disease of cucumber (*Cucumis sativus* L.), watermelon (*Citrullus vulgaris* Schard.), pumpkin and squash (*Cucurbita pepo* L., *C. maxima* Duch., and *C. moschata* Duch.), muskmelon (*Cucumis melo* L.), and certain other members of the Cucurbitaceae. Production of watermelon and fall cucumber crops in North Carolina is frequently seriously limited by this disease. Prior to 1949, when the anthracnose-resistant watermelon variety Congo was released, the disease was very destructive to watermelon (4). More recently, the widespread use of this, as well as of 2 additional anthracnose-resistant watermelon varieties, Fairfax and Charleston Gray, has resulted in smaller losses from the disease than were common earlier. In 1954 and 1955, an anthracnose disease was observed on resistant watermelon varieties at 4 scattered locations in North Carolina. Isolations from diseased leaves, fruits, and stems yielded a fungus indistinguishable from *C. lagenarium* (5). This suggested the occurrence of physiological races of *C. lagenarium*.

Several previous attempts to distinguish pathogenic races in *C. lagenarium* have been reported. In 1935, Rodigin (11) in Russia described 2 forms of *C. lagenarium*, alpha and beta, that were distinguishable by differential host reaction. Since his form beta produced typical fertile pycnidia in culture, it is suggested that he was working with different species rather than with 2 forms of the same species. Gardner (3) reported no infection following inoculation of several varieties of *Cucurbita* spp. with *C. lagenarium*, whereas all varieties of *Cucurbita* spp. inoculated by Layton (7) with a different isolate of *C. lagenarium* were susceptible. Layton recognized that his results differed from Gardner's but did not postulate possible race differences. In 1948, Parris (9) reported the existence of 2 pathogenically distinct strains of *C. lagenarium*. Two years later, however, he (10) suggested that these differences were associated with differences in virulence of cultures and that a second race of the fungus was not involved. In 1956, Goode (5) suggested that 2 or more physiological races of *C. lagenarium* were present in North Carolina.

The present investigation was directed towards pathogenic, cultural, and morphological comparisons of a large number of single-spore isolates of *C. lagenarium* from various cucurbit hosts in North Carolina and elsewhere in the Southeast, as well as an isolate from Wisconsin. Standardized inoculation techniques were developed for the differentiation of pathogenic races and for the evaluation of plants for resistance to the anthracnose disease. A preliminary report has been published (6).

MATERIALS AND METHODS.—Seedlings of various cucurbits, for inoculation studies, were grown in the greenhouse at 26°–32°C in a soil and sand mixture that had been disinfested with methyl bromide. These plants were usually inoculated in the 1- or 2-true-leaf stage. Disease-free fruits for inoculation studies were collected from isolated plots where no cucurbits had previously been grown for at least 4 years. At harvest, small fruits were placed individually in polyethylene bags and carefully handled to prevent injuries. Immature fruits were inoculated within 6 hours after

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harvest. Mature fruits were inoculated at varying intervals from immediately to several days after harvest.

For inoculation purposes, the fungus was grown in Petri dishes on a bean agar that contained 400 g of finely ground, cooked green beans (*Phaseolus vulgaris* L.), 20 g agar, and enough water to make 1 liter. Inoculum was prepared from Petri dish cultures held in polyethylene bags at 24°C for not more than 10 days after seeding. Conidia were washed off in distilled water and adjusted to a concentration of approximately 25,000 spores per ml. This spore suspension was sprayed on fruits or seedlings by means of an electric paint sprayer until all leaf or fruit surfaces were thoroughly wetted. After inoculation, seedlings were placed in a moisture chamber at 24°C at a relative humidity maintained at or near 100 per cent. Forty to 48 hours after inoculation, the plants were placed under a fluorescent light fixture with four 40-watt daylight bulbs and held for 7–10 days at the same temperature under constant light. Fruits were placed in large polyethylene bags for 36–48 hours after inoculation; the bags were then removed and the fruits held at 24°C.

Disease ratings on individual plants were made 7–10 days after inoculation as follows: 1) highly resistant or immune; 2) resistant, with a few small lesions; 3) intermediate, with a few well-formed leaf lesions and/or superficial stem lesions; 4) susceptible, with many large leaf lesions and/or deep stem lesions and usually with abundant sporulation; 5) highly susceptible, plants dead. Fruits were rated susceptible or resistant to the fungus.

Isolates used in this study were obtained from diseased plant material collected in the Coastal Plain and Piedmont regions of North Carolina. Sources of isolates included both anthracnose-resistant and anthracnose-susceptible varieties of watermelon, cucumber, cantaloupe, squash, and pumpkin. Isolates contributed by C. F. Andrus of South Carolina and by L. V. Busch of Wisconsin also were used in these studies. Cultures derived from single spores were made of all isolates prior to their use in inoculation studies.

TABLE 1.—*Relative susceptibility of seedlings of different varieties of watermelon, cucumber, and squash to Races 1, 2, and 3 of Colletotrichum lagenarium*

Differential host[a]	Average disease index following inoculation with indicated race[b]		
	Race 1	Race 2	Race 3
Watermelon			
Charleston Gray	2.1	5.0	1.1
Congo	2.3	5.0	1.1
Fairfax	2.3	5.0	1.1
Garrison	5.0	5.0	5.0
New Hampshire Midget	5.0	5.0	5.0
Cucumber			
Model	5.0	5.0	4.9
Palmetto	4.9	4.9	4.9
Squash			
Butternut	2.5	4.0	1.0

[a] Approximately 300 seedlings of each differential host were inoculated with isolates of each race.

[b] 1) Highly resistant or immune; 2) resistant; 3) moderately resistant; 4) susceptible; 5) highly susceptible.

RESULTS.—*Inoculation studies.*—North Carolina isolates from the anthracnose-resistant watermelon variety Congo and from the susceptible cucumber variety Marketer were used in the preliminary screening of seedlings of a large number of commercial varieties of watermelon, cucumber, squash, pumpkin, and cantaloupe. Host varieties were separated into groups according to their relative susceptibility or resistance to these isolates. Representative varieties from these groups were designated as host differentials and used later in screening additional single-spore isolates for pathogenic variation. The differential hosts selected included the watermelon varieties New Hampshire Midget, Garrison, Congo, Fairfax, and Charleston Gray; the cucumber varieties Model and Palmetto; and the squash variety Butternut.

When the standard inoculation technique described above was used, all isolates screened on seedlings of the differential varieties fell into 1 of 3 pathogenicity groups according to host reaction (Table 1). In the discussion of subsequent data, these 3 pathogenicity groups will be referred to as Races 1, 2, and 3. In Race 1 were placed those isolates highly pathogenic to seedlings of the Model and Palmetto varieties of cucumber and to New Hampshire Midget and Garrison watermelons, slightly to moderately pathogenic to Butternut squash, and nonpathogenic or nearly so to Charleston Gray, Congo, and Fairfax watermelons. Similar results were obtained when fruits were inoculated. Mature and immature fruits of Model and Palmetto cucumber and Garrison watermelon were highly susceptible; mature fruits of Fairfax, Congo, and Charleston Gray were immune, but their immature fruits (approximately 8 in. long or less) were susceptible (Table 2).

In Race 2 were placed a number of isolates obtained from vines, leaves, and fruits of the anthracnose-resistant watermelon varieties Congo and Charleston Gray. These were highly pathogenic to seedlings of all tested varieties except Butternut squash, which was rated as susceptible. All fruits inoculated, regardless of size or stage of maturity, were highly susceptible. These included Model and Palmetto cucumber and the watermelon varieties Garrison, Congo, Charleston Gray, and Fairfax.

Race 3 was represented in this study by a large number of single-spore isolates, all derived from the same parent culture. This culture was isolated by L. V. Busch from a cucumber fruit in Wisconsin. In screening tests, it was highly pathogenic to seedlings of Model and Palmetto cucumber and to New Hampshire Midget and Garrison watermelon. The anthracnose-resistant varieties of watermelon (Congo, Charleston Gray, and Fairfax) were highly resistant. Butternut squash seedlings were immune. Both mature and

immature fruits of Model and Palmetto cucumbers were highly susceptible; mature Garrison fruits were moderately susceptible. No disease symptoms developed on either immature or mature fruits of Congo, Fairfax, or Charleston Gray.

In subsequent tests, a plant introduction cucumber line, P.I. 163213, was inoculated with representative isolates of the 3 races. This cucumber line was highly resistant to Race 3 and highly susceptible to the other 2 races. The reaction of seedlings of Charleston Gray watermelon, Butternut squash, and *Cucumis sativus* (P.I. 163213) to isolates of Races 1, 2, and 3 are shown in Figure 1.

Cultural and morphological studies.—Cultural and morphological studies were made to determine whether the races could be differentiated by any characters other than pathogenic reaction of differential hosts.

Among representative isolates of the 3 races, 5 distinct cultural types could be distinguished on potato-dextrose agar at 24°C. These types were as follows: 1) essentially submerged mycelia, forming greenish-black to black colony and sporulating profusely with acervuli aggregated; 2) same as type 1 except little or no sporulation; 3) submerged dark mycelia and gray to pink-gray aerial mycelia with heavy sporulation in numerous but scattered acervuli (after 10 days the distance between acervuli was no closer than one-half the diameter of the conidial mass); 4) same as type 3 except little or no sporulation; and 5) mostly aerial mycelia varying from gray to pink and with sparse sporulation. No cultural type was unique to any one of the races. As much variation existed among isolates of the same race as among races.

Representative isolates of the 3 races were grown on potato-dextrose agar at each of 9 constant temperatures: 8°, 12°, 16°, 20°, 24°, 28°, 32°, 36°, and 40°C. All isolates grew slightly at 8° and made optimum growth at 24°; the maximum temperature at which growth was recorded was 32°. Sporulation by isolates of the 3 races was affected similarly by temperature. The minimum temperature at which sporulation was recorded was 16°, and the maximum was 28°. Sporulation was most abundant at 24°. Isolates within

TABLE 2. *Disease reaction of fruits of different varieties of watermelon and cucumber to Races 1, 2, and 3 of Colletotrichum lagenarium*

Differential host[a]	Stage of maturity[b]	Disease reaction following inoculation with indicated race[c] Race 1	Race 2	Race 3
Watermelon				
Charleston Gray	Immature	+	+	—
	Mature	—	+	—
Congo	Immature	+	+	—
	Mature	—	+	—
Fairfax	Immature	+	+	—
	Mature	—	+	—
Garrison	Immature	+	+	+
	Mature	+	+	+
Cucumber				
Model	Immature	+	+	+
	Mature	+	+	+
Palmetto	Immature	+	+	+
	Mature	+	+	+

[a] Approximately 60 fruits of each differential host were inoculated with isolates of each race.

[b] Immature watermelon fruits were 8 in. or less in length. Immature cucumber fruits were 3 in. or less in length.

[c] Positive (+) disease reaction was moderately severe to severe.

Negative (—) disease reaction indicated immunity.

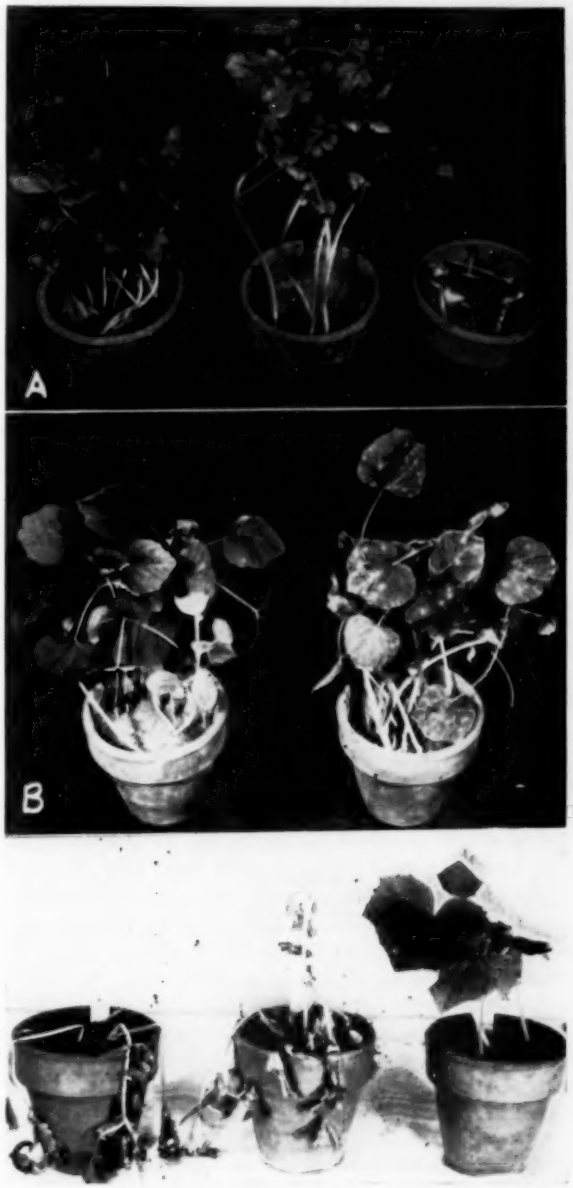

FIG. 1. Differential disease reaction to isolates of Races 1, 2, and 3 of *Colletotrichum lagenarium*. A) Charleston Gray watermelon plant inoculated with isolates of Races 3, 1, and 2 from left to right, respectively. B) Butternut squash inoculated with isolates of Races 3 and 1 from left to right, respectively. C) P.I. 163213 inoculated with isolates of Races 1, 2, and 3 from left to right, respectively.

a single race varied in cultural appearance and sporulation; however, no differences peculiar to a particular race were observed.

Single-spore isolates of the 3 races were grown at approximately 24°C on various media including 30 per cent V-8 juice (blend of vegetable juices manufactured by Campbell Soup Co.) agar, potato-dextrose agar, oatmeal agar, and green-bean agar. Sporulation of cultures varied from heavy to sparse on oatmeal, V-8 juice, and potato-dextrose agars. Sporulation was more frequently sparse on potato-dextrose agar than on any of the other media listed. All isolates from each race sporulated heavily to very heavily on green-bean agar. Although cultural characteristics of isolates varied somewhat on the different media, these differences were as great among isolates within races as among races.

The pH requirements for growth of isolates representing the 3 races were compared at 24°C on potato-dextrose agar containing a buffer prepared from glycine, citric acid, and potassium dihydrogen phosphate. All isolates responded similarly to hydrogen-ion concentration. The minimum pH level supporting growth was initially pH 3.6, but it shifted to pH 4.2 in all instances after 10 days' growth. Isolates of each race made maximum growth on media ranging from pH 5.4 to pH 6.8 initially. The final values of these media were approximately pH 6.9 for the 3 races. All isolates produced a trace of growth on media with an initial pH of 9.1, and little or no shift occurred. No isolate grew at a pH level above pH 9.1.

Studies of spore morphology also pointed out the very close similarities among isolates of the 3 races. A total of 500 spores of each race, including 250 from diseased plant tissue and 250 from potato-dextrose agar cultures, were measured. The minimum length in each instance was slightly less than 12 μ, and the maximum width slightly exceeded 6 μ. Other measurements fitted those given for the species by Gardner (3) and Mundkur (8). No isolates, irrespective of race, could be distinguished on this basis.

Resistance.—Sources of resistance to each of the 3 races were found among several plant introductions of *Cucumis sativus* (obtained from M. W. Hoover of Iowa). Seedlings of P.I. 163213 were highly resistant to Race 3 but highly susceptible to Races 1 and 2. Seedlings of P.I. 163217 were resistant to Race 2 but highly susceptible to Races 1 and 3. Seedlings of P.I. 175111, which were highly resistant to Race 3 in most cases, segregated for resistance to Race 2 and were susceptible to Race 1. Seedlings from 4 sources of P.I. 197087 varied greatly in their reaction to the 3 races. Some were highly resistant to Race 3 and highly susceptible to Races 1 and 2; others were resistant to Race 1 and susceptible to Races 2 and 3; others segregated for resistance to isolates of all 3 races.

Of commercial varieties of watermelon other than those listed in Table 1, Black Kleckley was susceptible to Race 2 and resistant to Race 1. It was not checked for reaction to Race 3. Among 354 watermelon plant introductions (obtained from E. James of Georgia) screened for resistance to Race 2, 20 showed some resistance. Approximately 50 per cent of the seedlings of line W-695 (obtained from J. R. Wall of South Carolina) had developed no symptoms at the end of 7 days, but a few of the survivors developed lesions at the soil line after 2 weeks; eventually all of these plants died. The extent of this resistance under field conditions has not been determined.

Discussion.—Isolates of each of the 3 pathogenic races, which are culturally and morphologically indistinguishable, are strongly pathogenic on susceptible cucumber and watermelon varieties and should be considered as 3 distinct pathogenic races of *Colletotrichum lagenarium*. The existence of 3 races can be clearly demonstrated by differential host reactions. Although 8 differential hosts were used in the present study, 3 of the 8 are adequate. These are Butternut squash (immune from Race 3 and susceptible to Races 1 and 2), Charleston Gray watermelon (resistant to Races 1 and 3 but highly susceptible to Race 2), and P.I. 163213 (susceptible to Race 1 and resistant to Race 3). It is suggested that the cucumber variety Model, which is highly susceptible to all 3 races, also be included among the differentials as a common host.

Inoculation techniques developed during the course of this study made it possible to distinguish clearly among the 3 races. Only on green-bean agar would all isolates sporulate profusely within 10 days. This made it possible to compare spore suspensions that were all young, of approximately the same age, and of similar concentrations.

The search for resistance in species of *Citrullus* and in *Cucumis sativus* gave some insight into the problems concerned in attempts to develop varieties of watermelon and cucumber resistant to anthracnose. Since some varieties within a species were resistant to certain races and susceptible to others, it seems probable that the plant breeder will have to work with resistances to specific pathogenic races of *C. lagenarium*. It is suggested that the differences in susceptibility reported by Barnes and Epps (1) and Busch and Walker (2) for certain plant introductions of *Cucumis sativus* might be explained on the basis that different races were involved.

Isolates of *Colletotrichum lagenarium* included in this investigation were obtained from a relatively small geographic area. Studies of the cucurbit anthracnose fungus from other areas might demonstrate the existence of additional physiological races of the fungus.

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PHYSIOLOGIC RACES OF THE DOWNY MILDEW FUNGUS ON SOYBEANS IN NORTH CAROLINA [1]

S. G. Lehman [2]

SUMMARY

Four new races of the downy mildew fungus (*Peronospora manshurica* (Naum.) Syd.) were obtained from collections of infected soybean seed harvested in North Carolina. The new races are designated 3A, 5, 5A, and 6. The reaction of 37 varieties of soybean (*Glycine max* (L.) Merr.) to each of these new races was determined. Ten of these varieties, Mukden, Richland, Illini, Roanoke, Acadian, C. N. S., Ogden, Palmetto, Woods Yellow 1, and S-100, are suggested as a set of differential varieties for separating all races thus far described in the United States.

Race 3A is regarded as a sub-race of Race 3 previously described by Geeseman from a collection made in Tennessee. It differs from Races 4, 5, and 5A by its ability to incite severe disease on C. N. S. and on Ogden and from Race 6 by its inability to infect Mukden and S-100. Races 5 and 5A differ from Race 4, previously described from North Carolina, by their ability to incite severe disease on S-100 and light to moderate disease on Illini, Ogden, and Palmetto, varieties immune from Race 4. They differ from Race 6 by their inability to infect Mukden and Richland and their ability to cause disease on Acadian.

Race 6 causes severe disease on the varieties Illini, C. N. S., and Ogden, which are immune from Race 4.

The soybean varieties Arksoy, Chief, Dorman, Dunfield, Otootan, Ralsoy, Rose Non Pop, Virginia Brown, and P. I. 157-463A show no symptoms after inoculation with any of the newly recognized races, whereas Cherokee, Mammoth Yellow, Mamotan, Rokusun, Tar Heel Black, Tokio, Volstate, and Jackson are moderately to highly susceptible to all of them.

In 1950, Geeseman [3] published descriptions of 3 physiologic races of *Peronospora manshurica* (Naum.) Syd. He designated these Races 1, 2, and 3 and showed that they can be distinguished by their reactions on Richland and any one of 5 other varieties. None of Geeseman's 9 collections came from North Carolina.

In 1953, Lehman [4] described a new race of *P. manshurica* that he had found in North Carolina. This race, which he called Race 4, fails to cause symptoms of mildew on any of the soybean varieties, such as Richland and Illini, that are parasitized by Races 1, 2, and 3 as described by Geeseman.

In 1953 and 1954, several additional cultures of *P. manshurica* were obtained from infected soybean seeds harvested in North Carolina. The purpose of this paper is to report the recognition of additional races and sub-races in these collections.

Methods.—The cultures of *P. manshurica* studied in these tests were obtained from soybean seeds encrusted with oospores. The infected seeds were planted in moist steamed soil and placed for germination in a cold greenhouse or in a constant-temperature room.

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[3] Geeseman, G. E. 1950. Physiologic races of Peronospora manshurica on soybean. Agron. J. 42: 257-258.

[4] Lehman, S. G. 1953. Race 4 of the soybean downy mildew fungus. Phytopathology 43: 460-461.

Temperatures were kept low, 50° to 60° F, to induce slow germination and slow growth of the seedlings. When the cotyledons of the emerged seedlings began to open, the seedlings were moved to a cool greenhouse to obtain slower and more normal elongation than occurs under artificial illumination. From 10 to 40 per cent of the seedlings from different lots of oospore-encrusted seed receiving this treatment developed symptoms of systemic infection. When the fungus within the primary leaves had developed to the sporulating stage, as indicated by a distinctly chlorotic green color of the invaded tissue, a single seedling from each collection was placed in a moist chamber in a room with a temperature of 50° to 60° F. Usually by the following morning an abundant crop of conidia was present on the infected leaves. To increase their number, these conidia were washed from the leaves and atomized onto young plants of the Roanoke variety. The cultures were maintained by transfer of conidia to young Roanoke plants at intervals of 10 to 14 days. To avoid mixing of races, plants infected with the different isolates were kept in different greenhouses. This appears to be an unnecessary precaution, however, if the temperature and humidity are such that free moisture does not collect on the leaves at night.

Approximately 40 named varieties or numbered selections of soybean were inoculated with each isolate of the fungus. The plants of each variety were grown in 6-in. pots. Leaves of susceptible varieties of soybeans increase in resistance rapidly with age, and differences occur both between and within varieties as to rapidity of seedling emergence. For these reasons an excess of seed was planted in each pot. By removal of the seedlings that emerged first and retaining for inoculation those that emerged later, the variation among varieties in age of leaves inoculated was greatly reduced.

Five plants were retained in each of 2 pots for inoculation. Inoculations were made as soon as the unifoliate leaves were three-fourths to fully unfolded and before the first trifoliate leaflets had opened. In some tests, a second inoculation was made when the first or second trifoliate leaf had opened. Two or more plantings of the soybean varieties were inoculated with each isolate of the fungus.

For inoculation, all varieties in a given test were set together in 2 replications on a greenhouse bench. The inoculum consisted of a water suspension of freshly harvested conidia diluted to contain 5–15 apparently viable conidia per low-power field of the microscope. This was applied to the plants as a mist from an electric sprayer in late afternoon and in quantities liberal enough to make escape of any plant quite improbable. The plants were set in an inoculation chamber equipped to keep some free moisture on the leaves. The temperature of the chamber was kept at 55 to 65° F for as much of the night as possible. The plants were removed to a greenhouse bench 18–20 hours after inoculation. Subsequently temperatures ranging from about 65° to 85° F were maintained.

Ratings of the amount and type of infection were taken 10–14 days after inoculation. Data for each of the 5 plants in each of the 2 replications were recorded for each variety in the test. The scale of infection ratings used by Geeseman in differentiating Races 1, 2, and 3 and by Lehman in describing Race 4 was found to be inadequate. Symptoms on different varieties were found to vary in a way indicative of different types of reaction. Thus, it became necessary to include in the scale symbols to indicate type of lesion as well as degree of infection. These symbols are explained in connection with Table 1, and the types of infection represented are illustrated in Figure 1.

Results and discussion.—The reactions of 37 soybean varieties to the new races and sub-races of the downy mildew fungus found in North Carolina are given in Table 1. The first 10 of these varieties may be used as differential varieties to separate all races and sub-races described thus far. For convenience of comparisons, the reaction of Race 4, as previously described by Lehman,[4] is included.

The isolate described as Race 3A was obtained from Ogden seed harvested at Weeksville, North Carolina, in 1952. The reaction of this race is similar but not wholly identical to that published by Geeseman for Race 3. Like Race 3, Race 3A incited severe disease on Illini and no visible symptoms on Mukden, Dunfield, Manchu, or Chief. On Richland, Race 3A caused lesions of a type designated by the symbol H in Table 1. These were faintly chlorotic, mostly less than 2 mm in diameter, and some of them were marked by necrotic centers (Fig. 1,E). Except for these necrotic centers, these H type lesions appear to be like the A_R type described and illustrated by Geeseman for the reaction of Race 3 on Richland. The similarities of lesion type on Richland and on the 4 other soybean varieties (Illini, Dunfield, Mukden, and Chief) that were common to the tests of Race 3 and this North Carolina isolate seem to indicate that the North Carolina isolate should be regarded as a sub-race of Race 3.

Race 3A differs from Race 4, previously found in North Carolina, by the production of severe mildew on Illini, C. N. S., and Ogden, varieties highly resistant to Race 4, and by its inability to infect Acadian, a variety moderately susceptible to Race 4.

The isolate designated Race 5 in Table 1 was obtained from Roanoke seed harvested at Rocky Mount, North Carolina, in 1952. Race 5 differs from Races 1, 2, and 3 of Geeseman in that it fails to infect Richland and produces disease of only moderate severity on Illini, a variety very susceptible to Races 1, 2, and 3. It incites only mild, moderately severe, or no disease on C. N. S., Ogden, Wabash, and Laredo, varieties highly susceptible to Race 3A. Also, it produces severe disease on S-100 and moderate infection on Acadian, varieties not infected by Race 3A.

Race 5 differs from Race 4 in that it produces mild or moderate mildew symptoms on Illini, C. N. S., Ogden, and Palmetto, varieties that are very resistant to Race 4, and incites severe disease symptoms on the

variety S-100, which is highly resistant to Race 4.

Additional isolates of the downy mildew fungus were obtained from soybean seed grown in 1953. One of these came from seed of the Roanoke variety grown at McCullers, North Carolina. Since this isolate incited disease symptoms on the differential varieties much like those incited by Race 5, it is regarded as a subrace of Race 5 and is designated Race 5A. Race 5A produced no disease on C. N. S., Woods Yellow, and Laredo, varieties moderately susceptible to Race 5.

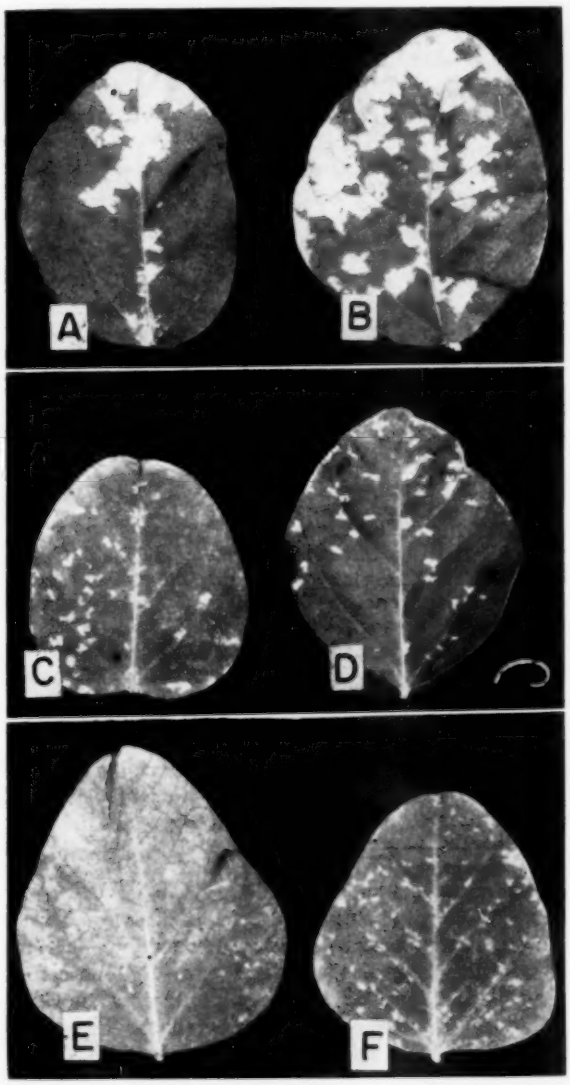

FIG. 1. Infected leaves showing lesion types represented by symbols used in Table 1. A) type 1—large, light green, chlorotic, angular lesions tending to coalesce, totaling 1–25 per cent of the leaf area. B) 2—like A, but totaling 26–50 per cent of the leaf area. C) N—yellow or yellowish green lesions, 2–4 mm diameter, minute reddish or brown flecks on some of them. D) F—light green, chlorotic lesions, 2–4 mm diameter, no reddish or brown flecks. E) H—faint to light green chlorotic lesions, 1–3 mm diameter, some having necrotic centers. F) D—faint to light green, 0.5–2 mm diameter, none having necrotic centers. All illustrations are approximately 5/7 natural size.

TABLE 1.—*Reaction of 37 varieties of soybean to races of Peronspora manshurica found in North Carolina*

Soybean variety	Reaction [a] to indicated race				
	3A	4 [b]	5	5A	6
Mukden	0	0	0	0	0,*N*
Richland	0,*H*	0	0	0	0,*N*
Illini	2,3,4	0	0,1	0,1	2,3,4
Roanoke	2,3,4	4	2,3,4	2,3,4	2,3,4
Acadian	0	1	1	0,*1*	0
C. N. S.	1,2,3,4	0	0,*H*	0	2,3,4
Ogden	2,3	0	0,*1*	0,1	1,2,3
Palmetto	0,D	0	*1*,2	0,*1*	F
Woods Yellow 1	N	1	1	0	N
S-100	0	0 [c]	1,2,3,4	1,2,3,4	F
Wabash	1,2,3,		0	0,F	1,2,3,4
Laredo	2,3,4		1	0	1,2,3,4
Arksoy	0		0	0	0
Armredo	1,2,3		1,2	1,2,3	1,2,3
Biloxi	0,1		1	0,1	0,1,2
Cherokee	2,3,4		2,3,4	2,3,4	2,3,4
Chief	0	0	0	0	0
Dorman	0		0	0	0
Dunfield	0	0	0	0	0
Imp. Pelican	0,D		1	1	F
Lincoln	2,3,4		0,1	0,*1*	2,3,4
Mammoth Yellow	2,3,4		2,3,4	1,2,3	2,3,4
Mamotan 6680	0,2,3,4		2,3,4	1,2,3	1,2,3,4
Manchu	0		0	0	0,F
Otootan	0		0	0	0
Ralsoy	0	0	0	0	0
Rokusun	2,3,4	3,4	3,*4*	2,3,4	
Rose Non Pop	0	0	0	0	0
Tar Heel Black	2,3,4		3,4	1,2,3	2,3,4
Tokio	1,2,3,4		2,3,4	2,3,4	1,2,3,4
Virginia Brown	0		0	0	0
Volstate	1,2,3		1,3,4	2,3,4	1,2,3,4
Jackson [d]	1,2,3	*1,2*	1,2,3	1,2	1,2,3
Perry	H		0,D	0,1	N
N49-2560 (Lee)	N	1,2,3	1,2	1,2,3	1,2,3
N48-1867	4	4	3,4	2,3,4	2,3,4
P.I. 157-463A [e]	0		0	0	0

[a] Significance of symbols indicating reaction of race on variety: 0—No symptoms; 1—Light green chlorotic lesions, angular, tending to coalesce, totaling 1–25 per cent of the leaf area (Fig. 1,A); 2—Like 1 but totaling 26–50 per cent of the leaf area (Fig. 1,B); 3—Like 1, coalescing, totaling 51–75 per cent of the leaf area; 4—Like 3, but totaling 76–100 per cent of the leaf area; D—Lesions chlorotic, faint to light green, diameter 1–2 mm, none having necrotic centers (Fig. 1,F); F—Lesion chlorotic, light green, diameter 2–4 mm, no reddish or brownish flecks (Fig. 1,D); H—Lesions chlorotic, faint to light green, diameter 1–3 mm, some having necrotic centers (Fig. 1,E); N—Lesions yellow or yellowish green, diameter 2–4 mm, minute reddish or brown flecks visible on some of them (Fig. 1,C).

Where 2 symbols are given and one is italicized, that in italics is the reaction found on more than half of the plants. If neither symbol is italicized, each symbol represents half of the inoculated plants.

[b] Described previously from North Carolina. Included for convenience of comparison.

[c] Omitted from previously published record because it was included only in Test 2 of Race 4. Now included as an extension of the reaction of Race 4. In light of behavior of other moderately resistant varieties in Tests 1 and 2 of Race 4, this variety would have given a reaction of 0, or at least not higher than 1, if it had been included in Test 1 of Race 4.

[d] Tested as N47-3470 in Test 2 of Race 4 and under the name Jackson for Races 3A, 5, 5A, and 6.

[e] U. S. Dept. Agr. Plant Introduction Number.

Race 5A appears to be about equal to Race 5 in ability to incite disease on the highly susceptible varieties, Roanoke and S-100, but is noticeably less virulent than Race 5 on the more resistant varieties C. N. S., Woods Yellow, and Laredo.

Two isolates obtained from separate lots of Ogden seed grown at Weeksville and Plymouth, North Carolina, in 1953 gave identical reactions when inoculated on the differential varieties. These isolates are designated Race 6 in Table 1. Race 6 differs markedly from races 4, 5, and 5A by the production of severe mildew on Illini, C. N. S., and Ogden, varieties unaffected or only moderately affected by these races. Race 6, like Race 3A, causes severe disease on Illini, C. N. S., Ogden, Wabash, and Laredo. It differs from Race 3A, however, by its ability to produce N type lesions on Mukden (Fig. 1.C) and F type lesions on S-100 (Fig. 1.D), varieties that showed no symptoms when inoculated with Race 3A.

Both amount of disease and lesion type are indicated by the symbols used in Table 1. As appraised by the writer in terms of varietal resistance to the races studied, the reaction represented by the symbol 0 indicates very high resistance or immunity; D and H, high resistance; N and F, moderate resistance; 1 and 2, low resistance or moderate susceptibility; and 3 and 4, high susceptibility. The reactions of the 37 soybean varieties included in Table 1 show that 9 varieties, Arksoy, Chief, Dorman, Dunfield, Otootan, Ralsoy, Rose Non Pop, Virginia Brown, and P. I. 157-463A are very highly resistant to or immune from all the newly recognized races and sub-races. Some of these may be of value as resistant parental varieties in breeding experiments. Acadian, Woods Yellow 1, Palmetto, Improved Pelican, Biloxi, and Perry show some resistance, whereas Cherokee, Mammoth Yellow, Mamotan, Roanoke, Tar Heel Black, Tokio, and Volstate are very susceptible to all of the new races and sub-races.

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A VIRUS DISEASE OF LUPINES CAUSED BY BEAN YELLOW MOSAIC VIRUS[1]

M. K. Corbett

SUMMARY

The principal virus disease of blue, yellow, and white lupines (*Lupinus angustifolius*, *L. luteus*, and *L. albus*, respectively) grown for seed and forage in the southeastern United States is caused by strains of bean yellow mosaic virus. The symptoms produced in diseased blue lupines are similar to those attributed to infection by pea mosaic virus in Australia and New Zealand. Diseased yellow lupines exhibit symptoms similar to those described as being induced by Lupinus virus 1 in Holland.

Experimentally, the host range of the viruses isolated was limited to the family *Leguminosae*. All tested species of the genus *Lupinus* were susceptible except 6 perennial species. The commonest type of isolate, LV1, withstood dilutions of 10^{-4} but not of 10^{-5}, a 10-minute exposure to a temperature of 58°C but not to one of 60°, and aging *in vitro* for 72 hours but not for 84 hours. The virus is transmitted in a nonpersistent manner by the aphids *Myzus persicae* and *Aphis medicaginis*. Positive immunological relationships with strains of bean yellow mosaic virus were demonstrated. The virus is transmitted through 6.2 per cent of the seed from diseased plants. The main field sources of the virus in northern Florida are the sweetclovers that grow as perennials.

Blue lupine, *Lupinus angustifolius* L., was released as a potential winter cover and green manure crop for the southeastern United States by Warner (24) in 1939. The recent trend has been to use sweet selections or low-alkaloid-content selections of *L. luteus* L. and of *L. angustifolius* for forage and seed crops. Decker (7) in 1950 reported a sap-transmissible virus of sweet yellow lupines in several of the lupine-growing areas of the Southeast. He observed that diseased plants yielded much less and set fewer seeds than did healthy ones. Weimer (26) reported 2 virus diseases of blue lupines that appeared in Georgia in epiphytotic form during the spring of 1949. Neill et al (19) described sore-shin, a virus disease of blue lupines, in New Zealand. Chamberlain (1) attributed the disease to infection by pea mosaic virus, and Norris (20) reported that both the bunchy top disease of L. *varius* L. and the sore-shin disease of *L. angustifolius* in Australia are caused by pea mosaic virus.

Cucumber mosaic virus has been reported by several investigators from Germany and New Zealand as causing a disease of blue lupine termed "lupine browning" (3, 14, 23).

Lupines grown in northern Florida and in southern Georgia for seed, forage, and green manure have exhibited symptoms characteristic of virus infection the

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past 2 years. This investigation was made to identify the virus or viruses involved, their properties, methods of spread, and means of oversummering.

SYMPTOMATOLOGY.—Since the symptoms produced in diseased plants of the 3 lupine species, blue, yellow, and white (*Lupinus angustifolius*, *L. luteus*, and *L. albus* L., respectively) have been described in detail elsewhere (5), they are described only briefly here.

The foliage of diseased blue lupine plants at an early stage of infection develops a characteristic bronze color, with a slight wilting. Some of the leaflets drop, and necrotic streaks develop on the stem. Necrosis of the stem usually is unilateral, and the apex of the plant tends to bend to the necrotic side, forming a shepherd's crook. The plant eventually dies.

Diseased yellow lupine develops vein clearing and a light mottle at an early stage of infection. Later, the mottle becomes more pronounced, and the leaflets of the new growth are usually narrow and malformed and tend to stand erect. Proliferation of the axillary buds often occurs, giving the new growth a bunchy appearance. If infection takes place during an early stage of development, the plant will be severely stunted but will remain alive throughout the growing season. The short flower racemes that are formed rarely if ever produce seed. Those pods that do form usually drop prematurely. Plants infected late in the growing season develop mosaic symptoms and various leaf deformations. Fruiting, in such cases, is usually normal on the central raceme. The lateral inflorescences that are developing while the plant is diseased are usually abnormal and result in pod abortion. Those pods that are formed and remain on the plant seldom contain many fully developed seeds.

Very few white lupines are grown commercially. Those that become infected develop a severe mottle, with various leaf deformations.

VIRUS ISOLATES AND METHODS OF INOCULATION.—Virus was isolated from diseased lupines of all 3 species and maintained separately in white lupine. White lupine (*L. albus* P.I. 202-527)[2] was planted in 4-in. pots of composted soil. Ten to 12 days after planting, the plants were thinned to 1 plant per pot; they were a suitable size for inoculation at that time. Unless otherwise stated, all inoculations were made by the gauze-pad method. Leaves were dusted with Carborundum, inoculated, and then rinsed with tap water. Inoculum consisted of expressed juice from 3-week-old systemically infected white lupine plants. Cross-inoculation experiments involving the 3 lupine species and the 3 virus isolates demonstrated that all 3 virus isolates caused identical symptoms in any 1 of the 3 species. All further work, unless stated otherwise, was with 1 isolate obtained from diseased yellow lupine. This isolate was designated LV1. For comparison with this isolate, 2 strains of bean yellow mosaic virus (BYMV) were used. One strain was obtained by Hagedorn (12) from peas, the other strain was obtained by Diachun (8) from red clover. These isolates were maintained in crimson clover (*Trifolium incarnatum* L.) and in red clover (*T. pratense* L.), respectively.

HOST RANGE.—Plants were inoculated when the primary leaves were fully expanded or when the first pair of true leaves was of suitable size. Five plants of each species were inoculated, and 1 plant of each was retained as a control. Most tests were replicated during different seasons of the year. Attempts were made to recover the virus from all inoculated plants by transfer back to the susceptible white lupine. Twenty-five species of the genus *Lupinus* and 48 selections of sweet yellow lupine obtained from Holland (15) via the U. S. Department of Agriculture were tested with isolate LV1 for a possible source of resistance or immunity to the disease.

A partial list of the plants tested and the comparative reactions of the 3 strains used are given in Table 1. The experimental host ranges of the 3 strains were limited to the family *Leguminosae*. Nineteen of 32 species tested of this family proved susceptible to isolate LV1. Thirty-three species of 9 nonleguminous families were tested and found to be immune.

Additional legumes that were found to be susceptible to isolate LV1 are the following: *Crotalaria mucronata* Desv.; sunn-hemp (*C. juncea* L.); sweetpea (*Lathyrus odoratus* L.); lespedeza (*Lespedeza striata* Hook & Arn.); white sweetclover (*Melilotus alba* Desr.); anual yellow sweetclover (*M. indica* All.);

TABLE 1.—*Symptoms induced by isolate LV1 from lupine and by 2 strains of bean yellow mosaic virus (BYMV) in some experimental differential hosts*

Host	Symptoms induced by indicated isolate[a]		
	LV1	BYMV	
		Pea strain	Clover strain
Lupinus albus	M	LLNL	M
L. angustifolius	NL	MD	NL
L. luteus	MD	MS	MD
Crotalaria spectabilis Roth.	LLN	LLN	M
Cassia tora L.	LL	LL	LL
Glycine max (L.) Merr. var. Dortch	—	M	—
Phaseolus vulgaris[b] L.	M	M	M
Pisum sativum L. var. Little Marvel	—	—	—
Perfection	—	—	—
Alaska	M	M	M
Trifolium incarnatum	M	M	M
T. pratense	M	M	M
Vicia faba L.	M	M	M
Vigna sinensis (L.) Endl. var. Blackeye	—	—	—
Datura stramonium L.	—	—	—
Nicotiana tabacum L.	—	—	—
N. glutinosa L.	—	—	—

[a] Letters indicate the following symptoms: M, mottle; N, systemic necrosis; L, lethal; LL, local lesions; D, leaf deformations; S, stunted; —, no symptoms of infection and no virus recovered.

[b] Varieties Great Northern, Bountiful, and Stringless Green Refugee.

[2] P. I. is an abbreviation for the U. S. Department of Agriculture Plant Introduction Number.

velvet bean (*Mucuna derringiana* (Bort.) Merr); serradella (*Ornithopus sativus* Link.); bean (*Phaseolus vulgaris* L.) var. Black Valentine, Burpee's Stringless, Kentucky Wonder, and Robust; pea (*Pisum sativum* L.) var. Alderman, Glacier, Miracle, Profusion, and Thomas Laxton.

Legumes found to be nonsusceptible are the following: peanut (*Arachis hypogaea* L.); pigeonpea (*Cajanus cajan* (L.) Millsp.); alfalfa (*Medicago sativa* L.); black medic (*M. lupulina* L.); mung bean (*Phaseolus aureus* Roxb.); Lima bean (*P. limensis* Macf.) var. Henderson Bush and Fordhook; Mungo bean (*P. mungo* L.); pea (*Pisum sativum* L.) var. Hundredfold, Loyalty, Notts Excelsior, Premium Gem, Pride, and Wisconsin Early Sweet; and Yard Long Bean (*Vigna sesquipedalis* (L.) Fruwirth).

There was no infection in *Gomphrena globosa* L., *Commelina nudiflora* L., zinnia (*Zinnia elegans* Jacq.), charlock (*Brassica kaber* (DC.) L. C. Wheeler), cucumber (*Cucumis sativus* L.), squash (*Cucurbita moschata* Duchesne), castor bean (*Ricinus communis* L.), pokeweed (*Phytolacca americana* L.), plantain (*Plantago ovata* Forsk.), pepper (*Capsicum frutescens* L.), or tomato (*Lycopersicon esculentum* Mill.).

All 48 selections of sweet yellow lupine were susceptible to infection by isolate LVI, as were 25 species of the genus *Lupinus* except for the following 6 perennials: *Lupinus arboreus* Sims, *L. cumulicola* Small, *L. longifolius* Abrams, *L. perennis* L., *L. polyphyllus* Lindl., and *L. villosus* Willd.

The similarity of the reactions suggest that the virus isolated from diseased lupines is a strain of BYMV. This differential host range includes all the plants reported by Goodchild (10) to be essential for the identification of BYMV strains.

PHYSICAL PROPERTIES.—Plants of white lupine were used both as source plants and as test plants. Source plants were used approximately 3 weeks after inoculation.

The numbers of plants that became infected following inoculation with various dilutions of juice diluted with distilled water were as follows: undiluted, 28 of 28; 10^{-1}, 26 of 28; 10^{-2}, 22 of 28; 10^{-3}, 4 of 28; 10^{-4}, 2 of 28; 10^{-5}, 0 of 28.

The thermal inactivation point was determined by heating 2-ml samples of infectious plant juice in small serological test tubes for 10 minutes at different temperatures and then cooling them immediately in tap water. The numbers of plants that became infected following inoculation with juice heated at various temperatures were as follows: unheated, 20 of 20; 40°C, 20 of 20; 50°, 20 of 20; 52°, 20 of 20; 54°, 18 of 20; 56°, 12 of 20; 58°, 6 of 20; 60°, 0 of 20.

Aging *in vitro* was at 20°C. The numbers of plants that became infected following inoculations with juice held for different periods were as follows: 24 hours, 27 of 28; 36 hours, 21 of 28; 48 hours, 6 of 28; 60 hours, 4 of 28; 72 hours, 2 of 28; 84 hours, 0 of 28.

The physical properties of isolate LVI from lupines compare very closely to those reported by Hagedorn (12) for isolate 1 from peas and with those reported by Goodchild (10) for strains of BYMV isolated from lupine.

VECTOR-VIRUS RELATIONSHIPS.—The aphids *Myzus persicae* (Sulz.) and *Aphis medicaginis* Koch are commonly found colonizing on lupines throughout the growing season. Adult apterous aphids of these 2 species were obtained from healthy field-grown yellow lupines and placed separately on healthy white lupines. Nymphs obtained from these were used to establish colonies used for all transmission studies.

In 1 series of tests, the 3 lupine species were compared as source and test plants. The aphids to be used were removed from the plants with a camel's-hair brush and placed in a vial for a 2-hour preacquisition starving period, after which single aphids were placed on the source plant and allowed an acquisition feeding period of 20–30 seconds. During the feeding periods, the aphids were observed with a hand lens and their feeding timed with a stopwatch. Following the acquisition feeding, single aphids were placed on test plants and allowed a 3- to 5-hour test feeding, after which all plants were fumigated with Nicofume and placed in the greenhouse.

Three sources of the lupine virus isolate LVI were used, i.e. 28-day systemically infected plants of yellow and white lupine and 11-day systemically infected plants of blue lupine. Young vigorously growing plants of white, yellow, and blue lupine were used as test plants. Control plants were handled as above except for the absence of an acquisition feeding.

The percentage transmission (Table 2) varied from 10 to 70, depending upon the source and test plant used. Plants of blue lupine were the poorest source plants, those of white lupine were intermediate, and those of yellow lupine were best. The 3 species appeared equally susceptible when used as test plants.

The length of virus retention by *M. persicae* was tested. Plants of white lupine were utilized both as source plants and as test plants. The aphids were handled as above except that they were allowed a test feeding period of 10 minutes on the first test plant

TABLE 2. *Comparative transmission of lupine isolate LVI by Myzus persicae from white, blue, and yellow lupines to test plants of the same 3 species*[a]

Source plant	No. of plants infected when indicated lupine was used as test plant[b]			
	White	Blue	Yellow	Totals
White lupine	13/20	8/20	8/20	29/60
Blue lupine	2/20	4/20	4/20	10/60
Yellow lupine	14/20	12/20	13/20	39/60
Totals	29/60	24/60	25/60	

[a] Aphids starved 2 hours, then given a 20- to 30-second acquisition feeding period followed immediately by a 3- to 5-hour test feeding.

[b] Numerator is number of plants infected and the denominator the number of plants used. In each test, 10 control plants remained healthy; aphids used for these were not given an acquisition feeding on the source plants.

and were then transferred serially to 4 additional plants for test feedings of 10, 10, 20, and 20 minutes, respectively.

The greatest length of virus retention was not more than 10 minutes, for in serial transfer only the first plant of each series became infected. Thus, the virus should be classed as nonpersistent (25) in *M. persicae*.

Because of difficulties in maintaining colonies of *Aphis medicaginis*, this species was not used for extensive studies except that it was found to be capable of transmitting the virus under the same conditions and procedures used with *M. persicae*.

IMMUNOLOGICAL RELATIONSHIPS.—A procedure previously described for determining relationships between strains of BYMV (6) was used. This test involves the use of plants of *Crotalaria spectabilis*, which react to infection by some strains of BYMV by the production of a mottle and to others by the production of local lesions and systemic necrosis. Isolate LV1 also induces necrosis.

One plant of *C. spectabilis* infected with isolate LV1 produced a side branch with a severe mottle. These mottled leaves were triturated and the expressed juice rubbed on a series of differential hosts (Table 1). The reaction was similar to that described for isolate LV1 except that the mutant caused a mottle in *C. spectabilis* whereas the original isolate still caused local lesions and systemic necrosis. This mutant designated LV2M, was used in subsequent cross-protection tests.

Twenty-five young plants of *C. spectabilis* were inoculated with the clover strain of BYMV, 25 were inoculated with strain LV2M, and 50 were maintained as healthy controls. Thirty days after inoculation all inoculated plants showed a strong chlorotic mottle. Twenty of these mottled plants and 20 healthy plants were then inoculated with the necrosis-producing pea strain of BYMV, and 20 of each inoculated with isolate LV1. Five mottled plants infected by the clover strain, 5 of those infected by the LV2M strain, and 10 healthy plants were maintained as controls.

The plants inoculated only with the necrosis-producing strains developed local lesions 4–6 days after inoculation and severe stem and tip necrosis 20 days after inoculation. The plants infected by the clover strain or by the LV2M strain before inoculation with LV1 and pea strains developed only an occasional local lesion in the dark green portion of the mottled leaves. No systemic necrosis developed. In similar tests, neither the clover strain nor strain LV2M protected against infection by tobacco ringspot virus.

These positive protection tests between the necrosis-producing LV1 and pea strain with the mottle-producing LV2M and clover strains indicate that the principal virus causing a mosaic disease of lupines is a strain of BYMV.

SEED TRANSMISSION.—From the amount of infection and the pattern of field spread it appeared that the LV1 strain was probably transmitted through the seed of diseased plants. Seeds were collected from field-grown diseased sweet yellow lupine plants. These plants were indexed for the presence of the virus. The seeds from these plants were planted in screened greenhouses that were sprayed periodically with parathion. Seeds of sweet yellow lupine variety Weiko III imported from Germany also were tested. All plants were observed over a period of 30 days. Plants suspected of being diseased were indexed for the presence of the virus on *C. spectabilis*.

On the 2521 plants grown from field-collected seed, 156 (6.2%) plants showed symptoms characteristic of virus infection. Such plants usually did not show symptoms until after the first 2 true leaves were formed. The subsequent new growth developed characteristic symptoms. Of the 4721 plants grown from seed of the Weiko III variety, none showed symptoms of virus infection. Tests for recovery of virus from some suspected cases in the Weiko III variety were negative.

FIELD SOURCES.—It is evident from the patterns of field spread that the virus must also come from some source other than plants grown from infected lupine seed. As lupines are normally grown during the months of November through April, such a source plant would be one that grows during the summer months. Evidence of the identity of 2 possible source plants was obtained from a blue lupine field in which there were more infected plants near 1 edge of the field than elsewhere. This edge was near a patch of sweetclover, *Melilotus alba* and *M. indica*, in which plants of both species showed vein clearing and a very light mottle. When expressed juice from these plants was rubbed on plants of the species listed in Table 1, the symptoms produced were similar to or identical with those induced by the LV1 and clover strains of BYMV.

Virus isolates from each species showed positive immunological relationships in plants of *C. spectabilis* with isolate LV1 and with the clover and pea strains of BYMV. The strain isolated from *M. alba* reacted similarly to the necrosis-producing strains whereas the isolate from *M. indica* reacted similarly to the mottle-producing clover strains in *C. spectabilis*.

Three hundred seedlings were grown from 1 diseased plant each of *M. alba* and *M. indica*. The seedlings developed no symptoms of virus infection nor could virus be recovered from random leaf samples.

DISCUSSION.—The results reported herein show that the principal virus disease of lupines in the southeastern United States is caused by a strain of bean yellow mosaic virus. The symptoms produced by diseased blue lupine plants infected with this virus are similar to those described by other workers for the sore-shin disease, which reportedly is caused by pea mosaic virus (1, 2, 4, 19, 20). Since it recently has been shown that BYMV and pea mosaic virus are serologically related (11), it would appear to be appropriate to attribute the sore-shin disease of blue lupines in Australia and New Zealand (4, 20) to a strain of bean yellow mosaic virus.

The Lupinus virus 1 described by Mastenbroek (16)

in Holland and the Florida virus induce similar symptoms, have similar physical properties, and are transmitted similarly. Presumably this virus is also a strain of bean yellow mosaic virus.

From the published descriptions, some of the 5 virus diseases described by Klesser (13) in South Africa would appear to be caused by strains of BYMV. Others would appear to be caused by strains of cucumber mosaic virus. It thus appears that the 2 principal virus diseases of blue, yellow, and white lupine in the world are caused by strains of BYMV and of cucumber mosaic virus.

The extensive leguminous host range of BYMV makes this virus a potential threat to many crops grown in the southeastern United States. Certain strains of BYMV have been isolated from nonleguminous hosts (9, 17). The high percentage of aphid transmission obtained experimentally indicates the relative ease with which the virus is transmitted from and to white or yellow lupine. Field observations have supported this in yellow lupine. The virus does not spread in fields of blue lupine to any great extent. The low percentage of transmission obtained when diseased plants of blue lupines were used as the source suggests the possibility of a low virus concentration due to the necrotic reaction. This, and the alkaloid content of the lupine (which may make them unpalatable to the aphids), may explain why the virus does not spread rapidly in fields of blue lupine.

Although the sweetclovers, *Melilotus alba* and *M. indica*, are annuals, they may serve as oversummering hosts of the lupine virus. They grow throughout the year in north Florida, making it possible to find plants of both species at all stages of development from seedlings to flowering at almost any time of the year.

Pea mosaic virus and cucumber mosaic virus apparently are not transmitted through the seed of diseased plants of blue lupine (1, 20, 23). Presumably this is because the hypersensitive reaction is lethal shortly after infection occurs. Mastenbroek (16) reported 5 per cent seed transmission of a virus causing the mosaic disease of yellow lupine in Holland. The extent to which BYMV is transmitted, 6.2 per cent, through the seed of diseased yellow lupine plants supplies a sufficient source of the inoculum to cause the entire field to be almost uniformly infected by harvest time.

It appears that the virus is not present in German-grown seed of the Weiko III variety. This variety is susceptible to BYMV and develops symptoms similar to those of diseased common sweet yellow lupine. Transmission of the virus through the seed of diseased plants of Weiko III sweet yellow lupine has not been tested. The virus has been reported from Germany (21, 22), but the plants may escape infection because of time of planting or because the diseased plants are rogued (16). In either case, the Weiko III variety of sweet yellow lupine grown in Germany offers, at present, a source of virus-free seed.

The only known possible sources of resistance or immunity are the 6 perennial species that appear to be immune. Up to the present, crosses between these species and the commercial blue, yellow, and white lupine have not been successful (J. R. Edwardson, Agronomy Dept., University of Florida, personal communication.)

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CHANGES IN AUXIN LEVELS IN SAFFLOWER HYPOCOTYLS INFECTED WITH PUCCINIA CARTHAMI [1]

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SUMMARY

A quantitative comparison has been made of the auxin content of ether extracts from healthy and from rust-affected safflower hypocotyls at various stages of development. Partial purification of the extracts was accomplished by re-extraction with isopropyl alcohol and subsequent paper chromatography. Bioassay of the chromatogram areas corresponding to the position of indoleacetic acid showed significantly greater growth-promoting activity in extracts from diseased tissue than in those from healthy tissue at all times during infection.

Extracts obtained from diseased tissue before signs or symptoms of disease indicated the presence of other growth materials, possibly precursors of indoleacetic acid, but identification was not possible. Visual observations of chromatograms sprayed with *p*-dimethylaminobenzaldehyde suggested a correlation between development of the hypocotyls and the presence of indole compounds.

Since excised safflower hypocotyls respond to indoleacetic acid by elongation in the same manner as intact hypocotyls respond to infection, the above evidence supports the hypothesis that higher auxin levels may be responsible for the initial increase in host growth and metabolism that occurs during infection by the rust fungus. Several mechanisms to account for the increased auxin levels and the possible significance of the findings for other obligate parasites are discussed.

Previous work has indicated that infection of safflower (*Carthamus tinctorious* L.) hypocotyls by *Puccinia carthami* Cda. has features that possibly are of general significance for studies of obligate parasites. Parasitic mycelium develops abundantly throughout the hypocotyl over a period of time suitable for detailed studies of the physiologic and biochemical sequence of events during infection. A notable characteristic is the close correlation between mycelial development and increased growth of the infected hypocotyl (6). Elongation is evident shortly before signs of the pathogen are noted and ceases when sporulation begins.

Studies of respiratory metabolism during pathogenesis (6, 7) suggested that the metabolic events can be separated into 2 phases. It was postulated that in the initial stages of infection the parasite induces a change in a key growth-controlling process of the invaded cells, causing an increased rate of host metabolism. As a consequence, the host furnishes the necessary substrates for growth of the parasite, and these substrates lead to rapid development of the fungus. As the mycelium increases in amount, its contribution to the total metabolic activity of the infected tissue gradually supplants that of the host. In the case of safflower rust (7), for example, and in that of bean rust (unpublished work), the evidence indicates that respiratory metabolism during sporulation is mediated by the hexose monophosphate shunt rather than by the Embden-Meyerhof pathway, which is of major importance in normal host tissue. The hypothesis can account for many of the physiological facts known about the behavior of obligate parasites in susceptible tissues (1). Although conjectural, it is useful as a model for the rational study of the physiology of infections caused by obligate parasites.

The marked elongation of infected safflower hypocotyl is typical of an auxin response, as defined in reference 5. The general tendency to implicate changes in indoleacetic acid (IAA) levels as a primary cause of variations in normal or abnormal growth should be tempered by the recent recognition of the possible importance in higher plants of growth inhibitors (4, 20), modifiers (21, 22) and previously unknown hormones (3, 11, 23). The complexities inherent in studies of growth-controlling processes are compounded by the difficulties (8) in the quantitative identification of the

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small amounts of compounds capable of inducing changes in growth habit. The experiments to be reported were designed to establish whether changes in ether-soluble auxins, particularly IAA, could account for the changes occurring in the early stages of infection of the safflower hypocotyl by *P. carthami*, with appropriate consideration of some of the above factors.

MATERIALS AND METHODS.—Inoculation and conditions for growth of safflower were as described previously (6), except that the temperature was slightly higher (19°–23° C). This resulted in more rapid development of inoculated and noninoculated hypocotyls than in previous studies. The percentage of infected hypocotyls was 95 or more in individual samples, but the amount of infected tissue per hypocotyl varied. Most hypocotyls were heavily infected. Since 2–3 hours were required to excise the hypocotyls in the large samples employed, they were placed in distilled water at room temperature until extraction was started. This procedure may have altered the absolute level of auxin somewhat, but since healthy and diseased tissue were treated identically, a comparison of the relative levels of auxin is valid.

Extraction.—A number of extraction procedures utilizing different solvents under different conditions of extraction were tested on fresh, frozen, or lyophilized tissue. The most consistent results were obtained by extracting fresh tissue for 3 successive 30- to 40-minute periods with fresh Mallinkrodt's anhydrous ether at 2° C. Five ml of prechilled ether was employed for each g fresh weight of tissue. In the initial extraction period, water to make 5 per cent by volume of the ether was added to aid extraction. After each extraction period, only the ether was decanted and it was not necessary to replenish the aqueous phase. Twenty g of young tissue was treated in this manner; larger amounts (up to 50 g) of older tissue were necessary as the auxin content of the hypocotyl declined. The extraction flasks were shaken vigorously at intervals.

The combined ether extracts were reduced to dryness at 50°–60° C under reduced pressure in a special flask constructed with a basal depression to facilitate quantitative removal of liquids. A known volume, never greater than 1.0 ml, of isopropyl alcohol, was used to extract auxin from the thin film of dried residue. The use of isopropyl alcohol has 2 main advantages over the customary reduction of ether to a small volume: 1) some ether-soluble materials are not dissolved by isopropyl alcohol and hence do not interfere with assay and 2) the final volume of solution is known more exactly and there is less change in volume due to evaporation during subsequent handling.

Aliquots (100 λ) were transferred to filter paper and dried in a stream of warm air before bioassay and/or paper chromatography.

The possibility that the ether or alcohol might contain materials that interfere with the assay was checked by reducing twice the maximum amount of ether employed and testing the residue in isopropyl alcohol for activity. Bioassay with oat coleoptiles in the presence and absence of indoleacetic acid showed that the materials used for extraction had no effect on coleoptile growth.

Bioassay.—Filter paper sections spotted with the original extract or obtained after chromatography were placed in Stender dishes, in triplicate, with 3.0 ml of distilled water. Oat coleoptiles of the variety Garry, grown in the dark at 25° C, were selected when 1.5–2.5 cm long. Sections 7 mm in length were excised from a point 4 mm below the tip under diffuse red light at 20° C by means of a cutter similar to that employed by Bentley (2). The sections were placed for 2–4 hours in 500 ml of water containing 1 ppm manganese as recommended by Nitsch and Nitsch (12). After they were rinsed with distilled water, 10 sections were placed in each Stender dish and kept at 25° C for 18–24 hours.

The length of the sections was measured to the nearest mm with a translucent, flexible ruler after a 10-fold magnification of the coleoptile image by projection on a wall screen. Since the images ranged from 80 mm to 130 mm, this procedure lowered the error of measurement to less than 2 per cent, and the flexibility of the ruler eliminated errors owing to slight curvatures of the sections. Growth activity of the extracts was expressed as "percentage elongation," i.e. the elongation of the treated sections expressed as a percentage of that of 3 control replicates incubated in distilled water. Modifications, similar to those outlined by Nitsch and Nitsch (12), were tested but offered few advantages over the procedure outlined. Phosphate buffers as incubation media are desirable, but we observed that some lots of commercial potassium phosphate contain inhibitory substances at concentrations below the inhibitory activity of the buffer alone.

Since the sensitivity of oat coleoptiles to a given concentration of IAA varies with age and pretreatment, a standard curve of 6, 12, 24, 47, 93, and 187×10^{-3} μg of IAA was run with each assay. Two replicates of each concentration were used to calculate the constants for the equation, percentage elongation = a ($-$ log μg IAA) + b, by the method of least squares. The elongation of sections treated with extract was converted to IAA equivalents (IAE). We use this terminology in expressing results because of the absence of definitive chemical proof that the growth-promoting materials are actually IAA. Careful adherence to the procedure for selection and sectioning of coleoptiles gave satisfactory reproducibility in the growth response at the concentrations of IAA employed. The lowest concentration produced 3 per cent greater elongation than did the controls, whereas 187×10^{-3} μg IAA averaged 20 per cent. Although higher concentrations could be employed, elongation in the presence of extracted auxin fell within this range. Extraction and assay for each sample were completed within a 36-hour period in order to minimize changes in the extracts.

The statistical significance of the differences in elongation among controls, healthy-tissue extracts, and diseased-tissue extracts was estimated by standard *t*-tests.

TABLE 1.—*Auxin activity in 4-fold dilutions of extracts from safflower hypocotyls infected with Puccinia carthami*

Mg of tissue tested [a]	μg indoleacetic acid equivalents ($\times 10^{-3}$) expressed on indicated basis	
	Actual sample tested	Per g fresh weight
60	7.0	118
235	8.8	36
940	12.0	13
3750	29.0	7.9

[a] Extract from 25 g of tissue was made to 1.0 ml; 0.15 ml of the original extract and of dilutions of ¼, 1/16, and 1/64 were assayed in triplicate.

In Table 2, differences between control elongation and either healthy- or diseased-tissue extracts were significant at the 1 per cent level whereas differences between healthy- and diseased-tissue extracts are given in the table. In the series of extractions represented in Figure 1, all the differences between diseased and healthy tissues in IAA are significant at the 1 per cent level; however, the differences between healthy-tissue extracts and the controls are not significant. Although the elongation was greater than that of the controls, it was less than the elongation obtained at the lowest level of IAA in the standard curve. Since it is impossible to extrapolate with confidence below the minimum value of the standard curve, these figures should be considered maximum auxin concentrations. Data taken on fresh weight and dry weight per hypocotyl permitted the expression of the auxin equivalents of the extract aliquot in a number of different units.

Paper chromatography.—Preliminary dosage-response data (Table 1) suggested the presence of growth-inhibiting, as well as growth-promoting, substances in extracts of both healthy and diseased tissues. In this experiment, 25 g of tissue was extracted and made to 1.0 ml with isopropyl alcohol; 0.15 ml of the original extract and of dilutions of ¼, 1/16, and 1/64 were assayed in triplicate. Auxin activity did not increase in direct proportion to the amount of tissue tested (column 2), and the apparent activity per unit of fresh weight increased with dilution (column 3). In the absence of interfering materials, the latter quantity should be constant.

Partial purification of IAA was achieved by ascending chromatography on Whatman No. 1 filter paper (21 × 20 cm) in a mixture of isopropyl alcohol, ammonium hydroxide, and water (10:1:1 by volume) overnight in the dark at 25° C. The final distance of development was approximately 18 cm. Two spots of extracts from both healthy and rust-affected tissue were placed at the origin by multiple spotting of 10-λ aliquots until a total of 100 λ at each spot was obtained. This amount resulted in overloading, particularly with lipid components of the extract, and consequent fuzzy resolution, but it was necessary in order to obtain IAA levels suitable for bioassay. Two μg of IAA added to a single spot each of healthy-tissue and diseased-tissue extracts and a fifth spot of IAA alone were included on each chromatogram.

After development, the paper was cut into 5 longitudinal strips, and the 3 strips containing added IAA were sprayed lightly with 1 per cent *p*-dimethylaminobenzaldehyde (PAB) in 1 N HCl. Pure IAA had an R_F of 0.36–0.40 in different tests and produced a blue color as reported by Sen and Leopold (18). IAA added to extracts was consistently slower moving (R_F = 0.30–0.37) than when alone and produced a pink-blue color with PAB. The latter effect apparently is a result of the presence of other materials since the area showed a strong fluorescence under a Mazda BH4 100-watt ultraviolet source. Although IAA is reported to show fluorescence (18), it has not been observed under our conditions when 2.0 μg of IAA in freshly prepared solutions was used. Chromatogrammed extracts without added IAA showed equally strong fluorescence even though the amount of natural IAA was not sufficient to develop color with PAB. Furthermore, the fluorescent material could be separated from IAA by development in butanol-ethanol-water (4:1:1), and the IAA added to extracts then produced the same shade of blue as did pure IAA.

The IAA content of extracts was determined on sections of the 2 untreated strips. In order to ensure that IAA was included in the section, the paper was cut approximately ½ cm above and below the area corresponding to the 2.0 μg of added IAA located on the sprayed strips. The growth-promoting activity in this area was much higher than that of similar amounts of unchromatogrammed extracts. Growth-inhibitor activity was correlated with a red oil, which moved near the solvent front (see Fig. 2, 3).

RESULTS.—In Tables 2 and 3 are recorded the patterns of growth and auxin levels, respectively, for healthy and rust-affected safflower hypocotyls collected at successive intervals after inoculation.

At 8 days there were no visible indications of infection or gross differences in size. From the eighth to

TABLE 2.—*Growth patterns of healthy and of rust-affected safflower hypocotyls*

Days after planting [a]	Kind of tissue	Length (cm)	Fresh weight (mg)	Dry weight (mg)	Infection
8	Healthy	1.43	45	3.0	
	Diseased	1.42	44	2.8	Not visible
10	Healthy	1.60	47	2.7	
	Diseased	1.99	62	3.2	Mycelial
13	Healthy	1.92	58	2.8	Initial
	Diseased	2.41	71	3.7	pycniospores
16	Healthy	1.89	54	2.6	Initial
	Diseased	2.46	81	3.9	urediospores

[a] Safflower seed were placed in rag dolls at 25° C for 24 hours. One half of the germinated seeds were then dusted with a talc-teliospore mixture. Inoculated and noninoculated lots of seed were planted in alternate flats of sand in a greenhouse maintained at 19°–23° C. Samples representing 25–50 g of hypocotyl tissue (after removal of the roots and leaves) were randomly selected for the growth measurements and auxin extraction.

thirteenth day, elongation occurred most rapidly in the diseased hypocotyls, and the fresh weight increased markedly. Mycelial development was evident during this time. Growth of both normal and diseased hypocotyls had ceased when sporulation was initiated. As is shown in Table 3, there are significant differences in auxin level throughout rust development, including hypocotyls assayed on the eighth day, when no visible symptoms or signs of infection were apparent.

Although the extracts were prepared from equal amounts of fresh weight of healthy and of infected tissue, the data in Table 2 clearly show that, because of differences in rates of elongation and final size, comparison on a fresh-weight basis may not be valid. Similar arguments have been presented previously in connection with measurements of respiratory rates.

Such arguments are even more applicable in the case of auxin levels, since it is held generally that auxins (or precursors) are produced for the most part at specific sites (meristematic tissue) and then distributed to the organ undergoing development. In the case of normal safflower hypocotyl, as with other species, removal of the hypocotyl tip prevents normal growth, an indication that the tip is the primary source of auxin. Although the site of synthesis of auxin in diseased tissue is uncertain (see Discussion), it would appear more accurate to compare healthy and diseased tissue on an organ basis than on any other. On the 16th day, for example, there was a 7-fold difference between healthy and diseased tissue on a fresh-weight basis; however, since diseased hypocotyls weighed approximately 50 per cent more than did healthy hypocotyls, there were fewer hypocotyls represented per unit fresh weight in the healthy than in the diseased samples. On a hypocotyl basis, therefore, the difference in auxin concentration was 10-fold.

During the development of the parasite the relative auxin level of infected tissue increased more than did that of control tissue (Table 3). The reason for the pronounced increase in both healthy and diseased tissue on the 13th day is not known but may have been the result of auxin movement into the hypocotyl from the first true leaves, which emerged at that time. Both healthy and diseased tissue show decreased auxin levels after a peak that was reached on the 13th day, and the decline occurred at approximately the same rate.

Similar trends are shown for another series in Figure 1, except that the differences were greater than in the previous experiment, and a decline in auxin level in diseased tissue was not observed. Despite their greater content of IAA, diseased hypocotyls were only 23 per cent longer than healthy ones in contrast to a 30 per cent difference in the first experiment. It is possible that failure to obtain greater growth in diseased tissue is owing to the inhibitory effects of auxin, as indicated by Pilet's (15) observations on rust-affected leaves of *Euphorbia cyparissias* L. We frequently have observed growth inhibition in heavily infected bean leaves, whereas moderate infections increase leaf area.

Partial confirmation of the role of IAA in elongation of safflower hypocotyls was obtained *in vitro* with 1-cm sections of dark-grown hypocotyls excised 5 mm from the tip. Elongation of sections was a logarithmic function of added IAA over a range from 0.01 to 1.0 ppm, with a maximum of 50 per cent elongation for sections from hypocotyls 2–5 cm long when excised. Sensitivity to added IAA decreased with age of hypocotyls.

Although elongation appears to be controlled by IAA in a manner similar to its role in growth of other tissues, it was of interest to determine if the extracts contained additional materials influencing cell elongation, particularly as a consequence of infection by *P. carthami*. Special emphasis was placed on tissue in the early stages of infection in order to detect possible differences in precursors of IAA.

Chromatogram sections 2 cm in width were cut serially, starting 1 cm below the starting line of ascending columns, and tested with oat coleoptiles (Fig. 2). Since the dosage response of oat coleoptiles to growth materials other than IAA was not known, the results are expressed as percentage elongation of the original length. The dotted line C represents elongation of control sections in water, whereas the shaded areas correspond to the activity of sections utilized for the data in Table 3. Chromatograms of the 8-day samples

TABLE 3.—*Auxin*[a] *levels in healthy and rust-affected safflower hypocotyls*

Days after planting[b]	Type of tissue	μg indoleacetic acid equivalents expressed on the indicated basis		
		Per g fresh weight	Per g dry weight	Per hypocotyl
8*	Healthy	6.5×10^{-3}	9.7×10^{-2}	2.9×10^{-4}
	Diseased	14.0×10^{-3}	22.0×10^{-2}	6.2×10^{-4}
10*	Healthy	15.0×10^{-3}	26.0×10^{-2}	7.0×10^{-4}
	Diseased	30.0×10^{-3}	61.0×10^{-2}	19.0×10^{-4}
13**	Healthy	5.2×10^{-3}	10.0×10^{-2}	2.9×10^{-4}
	Diseased	18.0×10^{-3}	35.0×10^{-2}	13.0×10^{-4}
16**	Healthy	2.1×10^{-3}	4.2×10^{-2}	1.1×10^{-4}
	Diseased	14.0×10^{-3}	28.0×10^{-2}	11.0×10^{-4}

[a] Assay of chromatogram areas corresponding to indoleacetic acid.

[b] Single and double asterisks indicate that the differences between assays of tissues obtained on the indicated day are statistically significant at the 5 and 1 per cent levels, respectively. These data obtained from the same planting of safflower used in obtaining the data of Table 2.

represented 2.0 g of tissue, corresponding to 45 hypocotyls, for both healthy and diseased tissue. Three g of tissue was used at 13 days, which corresponded to 50 healthy, or 40 diseased, hypocotyls.

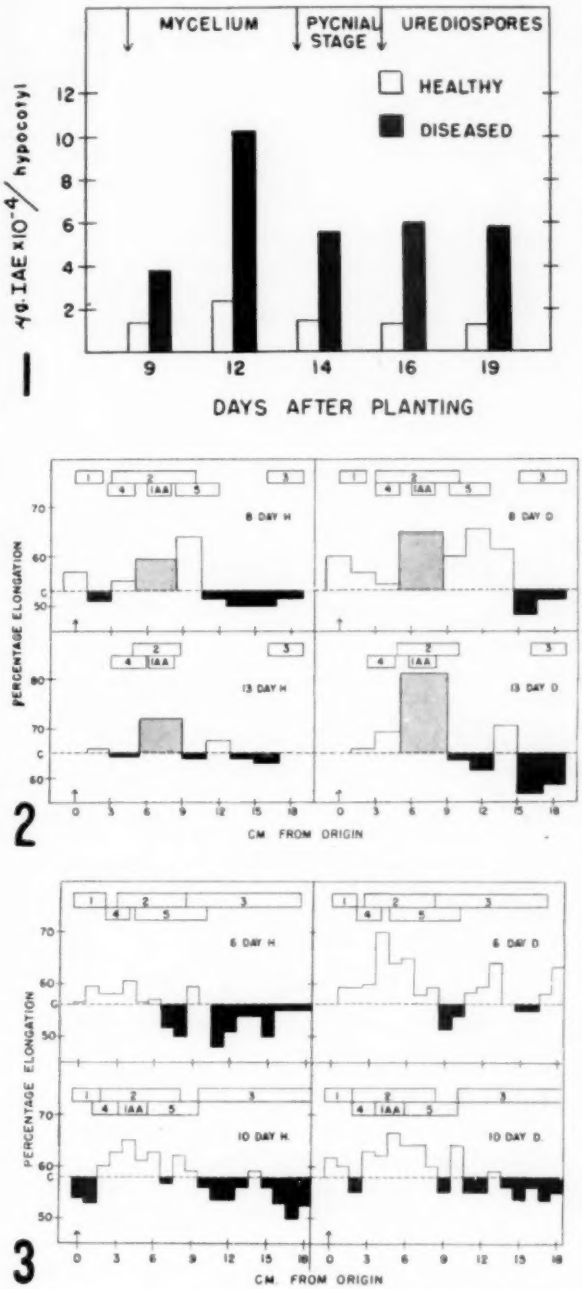

FIG. 1-3. FIG. 1. Auxin levels in healthy and rust-affected hypocotyls of safflower during development of the parasite *Puccinia carthami*.—FIG. 2. Growth activity of serial sections from chromatograms of ether extracts from healthy (H) and rust-affected (D) hypocotyls 8 and 13 days after planting.—FIG. 3. Growth activity of serial sections from chromatograms of ether extracts from healthy (H) and rust-affected (D) safflower hypocotyls. Extracts prepared from a different lot of plants than in Figure 2 and at 6 and 10 days after planting.

At 8 days, before visible signs of disease had appeared, several areas other than that corresponding to IAA had growth-promoting activity. The diseased-tissue chromatogram showed 2 sections (11–13 cm and 13–15 cm) of increased growth activity that do not appear in the healthy-tissue extract. Five days later, only the section in which IAA was located produced appreciable elongation, despite the fact that a greater fresh weight was employed in preparing the chromatogram. Since the neutral auxins would be located in the general areas showing activity in the early sample, attempts to characterize these areas by spraying with PAB were made. The rectangles in the upper row of such chromatograms (Fig. 2) delineate areas capable of identification without spraying. These appear as follows: 1) light yellow; 2) fluorescent under ultraviolet; 3) red-orange pigment associated with safflower oils. The lower rectangles are spots produced by PAB as follows: 4) light blue-gray; 5) intense blue-purple; IAA) pink-blue color produced by 2.0 μg of added IAA. There was never sufficient natural IAA in the extracts to undergo reaction with PAB.

The blue-purple area designated as 5 has been observed to be correlated consistently with development of the hypocotyl. It is the major spot produced by PAB in chromatograms from young hypocotyls and is still more prominent in those from rusted tissue, but it gradually disappears from the chromatogram with maturation of the tissue. In this series, for example, it was not discernible on the 13th day.

Since the number of hypocotyls represented by the chromatograms of Figure 2 varied with date of sampling and with kind of tissue (rusted and normal), a separate series based on identical number of hypocotyls for 2 different sampling times was undertaken. The chromatogram represented 65 hypocotyls of both healthy and diseased tissue. In addition, the size of strips tested was reduced to 1 cm in order to obtain better resolution of growth-active and growth-inactive areas. The first sample was taken 6 days after planting and the second sample at 10 days (Fig. 3). The general observations made were similar to those made previously. Young diseased tissue showed additional areas of growth-promoting activity not present in healthy tissue or associated with IAA. In later samples, only areas corresponding to the location of IAA were active in the coleoptile test.

Two features of the chromatogram should be noted. In the 6-day sample, the spots designated 4 or 5 were so large and intensely colored that the area occupied by added IAA could not be located with precision. In the 10-day sample, 65 hypocotyls of diseased tissue corresponded to 5.0 g fresh weight as compared to only 4.5 g of normal tissue. In both types of tissue, but particularly with rust-affected tissue, these large amounts resulted in streaking and poor resolution of the chromatogram.

DISCUSSION.—The results of the present investigation are consistent with the hypothesis that the increased

metabolic activity of rust-affected safflower is a consequence of a primary disturbance of hormonal balance during the initial stages of infection. The increased growth that occurs during mycelial development of the parasite could result from 2 principal mechanisms: 1) removal of a growth inhibitor or 2) actual increase in growth-stimulating substances in the invaded tissue. The first possibility is not eliminated in this study, chiefly because only ether-soluble compounds have been examined, but the inhibitors present in this fraction appear to be associated with characteristic safflower oils found in both healthy and diseased tissue.

The pronounced increase in growth-promoting activity of diseased tissue before any visible symptoms or signs of infection indicate that the second alternative is the principal one. The consistent association of growth activity with the chromatogram area corresponding to IAA is strong evidence that this compound is the hormone involved. It should be emphasized, however, that the complex nature of ether fractions, in addition to the present scant information on the role of other growth hormones (such as gibberellic acid) in higher plants and fungi, requires caution in the interpretation of results obtained with extracts partially "purified" by chromatography. This is particularly true when bioassay, rather than chemical determination, is employed. The *in vitro* response of safflower hypocotyls to synthetic IAA is supporting evidence for IAA participation. Also suggestive is the presence in the extracts, especially in those of infected hypocotyls, of other compounds reacting with indole reagents.

The quantitative evidence, however, does not give an answer to the more fundamental question as to the mechanism responsible for the increased auxin levels. Since steady-state levels of auxin result from equal rates of formation and destruction, the initial increases in infected tissue may be explained by 1) increased rates of synthesis or 2) "protection" of auxin by an inhibitory action on the degradation of the molecule (10). If the first alternative is correct, it raises the problem of establishing whether the higher levels result from direct production of auxin by the parasite or by indirect effects on the normal auxin-producing system of the host.

The detection of other growth-promoting areas on chromatograms of ether extracts from diseased hypocotyls may be attributed to precursors of IAA during the early development of the pathogen. The rates of migration of these materials in the irrigating solvent used are similar to those of postulated neutral precursors such as indoleacetonitrile and tryptamine (4, 9, 18), but the absence of data on R_F values for pure compounds in safflower extracts prevents a positive comparison. Further complicating the interpretation of chromatograms are the uncertainties surrounding the exact pathways of IAA metabolism, even in species studied in detail (8, 9), and the possibility that alternative pathways may be involved in pathogenesis. Consideration of some of the difficulties mentioned previously in the section on results indicates that careful kinetic analysis of IAA synthesis and degradation in both healthy and diseased tissues, with utilization of methodology other than paper chromatography, will be necessary in order to determine the origin of the auxin found in diseased tissue.

Another possibility to be considered is suggested in the work of Shaw and Samborski (19), who have shown accumulation of a number of metabolites, including IAA, at infection sites. Two arguments against metabolic accumulation in the present study are the high concentrations of auxin found before elongation, when metabolic rates of infected safflower hypocotyls are the same as those of normal hypocotyls, and the evidence for other growth-active materials in the earliest stages investigated. Each of the suggested mechanisms of increased synthesis, retardation of breakdown, and accumulation of auxin, may be found to be predominant at different times during parasite development.

It has been suggested (6) that alteration of the metabolic processes concerned with growth may be a general method for the successful establishment of obligate parasites. There are many examples of additional host growth induced by rust fungi. Particularly outstanding are the fungi causing the blister and gall rusts of conifers, cedar-apple rust, rose rust, and ash rust. In these instances, considerable areas of host tissue are invaded. The relatively localized area and short time period required for mycelial development may be a major reason why pronounced growth abnormalities commonly are not observed for rust fungi on foliar tissue. Yarwood and Cohen (24) have shown hypertrophy of cells infected with the bean rust fungus, and Rice (16) has pictured a similar response with corn rust. Yarwood and Cohen also established that leaf area was increased by infection by rust fungi but that the extent of enlargement was a function of the number of pustules per unit area. Heavy infection actually caused growth inhibition; a similar effect is common for high concentrations of auxin.

Unpublished data by Bell and Daly, on intact bean plants in contrast to detached leaves as used by Yarwood and Cohen, substantiate the findings of Yarwood and Cohen, but it also was noted that young immature tissues give more striking responses than do older tissues. Photographs of *Euphorbia cyparissias* L. infected with *Uromyces pisi* Pers. illustrate the influence of tissue type on the obtained response (15). Heavily infected leaves are smaller than normal, but the apparently disease-free stems of infected plants show marked elongation. This may be a result of auxin movement into a responsive tissue. In contrast, Sempervivens leaves infected with *Endophyllum sempervivi* Lev. were approximately 50 per cent longer than normal (14). Leaf areas were not given.

Whether the same materials are involved in the disturbance of the growth habit of different tissues and with diverse rust fungi is a question for further works. Various compounds other than IAA, such as adenine and kinetin (17), have been implicated in leaf expansion. Pilet (14, 15) has presented data indi-

cating large (100- to 1000-fold) differences between rust-affected and normal Euphorbia and Sempervivens leaves in the content of materials promoting curvature of the oat coleoptile. Apparently only crude ether-extracts were assayed, hence the nature of the materials and the role of nonauxin compounds in the coleoptile curvature can not be evaluated. The quantitative accuracy of Pilet's determinations also is subject to question especially since the range of IAA equivalents in his standard curve was extremely wide. The assay gave only a 20° curvature for concentrations from 10^{-13} to 10^{-3} moles of IAA (13). It should also be noted that the response from 10^{-7} to 10^{-3} moles IAA (infected leaves contained 10^{-4} moles of IAA) was measured by an inhibition of curvature without consideration of naturally occurring inhibitors in the extracts tested.

Shaw and Samborski (19) have reported excess IAA in rusted and mildewed leaves of wheat and barley, but they gave no details on analytical methods, stage of parasite development, or the magnitude of the differences. Without implicating auxins, they conclude that their results on accumulation of metabolites at infection sites are consistent with the hypothesis "that a diffusible substance (or substances) produced at incipient infection . . . . . stimulates the metabolic activity of the host tissue in the environs of the infection." The present and previous papers on safflower rust support the hypothesis that changes in the level of naturally occurring hormones bring about the metabolic stimulation of host cells in the early stages of infection, resulting in abundant substrates for initial mycelial development of the parasite.

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TEMPERATURE AND TEMPERATURE-LIGHT EFFECTS ON THE CONCENTRATION OF SQUASH MOSAIC VIRUS IN LEAVES OF GROWING CUCURBITS[1]

John B. Bancroft

SUMMARY

The concentration of squash mosaic virus in leaves of pumpkin and squash plants grown under various conditions of light and temperature was measured spectrophotometrically. Although the virus initially increased fastest at higher temperatures, it reached higher concentrations for a longer period of time at 15.5° and 21.0° C than at 26.5° and 32.0° in both hosts during 20-hour days and under 740 foot-candle light intensity. Experiments in which photoperiods of 8 and 20 hours at 740 foot-candles and temperatures of 15.5° and 26.5° C were used revealed that the virus made the greatest initial increase at high temperatures during long days. No constant trend was noted at low temperatures. Subsequent assays of both hosts at high temperatures revealed no difference at the different photoperiods, whereas at low temperatures the leaves of short-day plants contained much more virus than did those of long-day plants or those of either photoperiod at high temperatures. The same pattern was found when light intensities of 220 and 740 foot-candles during 20-hour photoperiods were studied at temperatures of 15.5° and 26.5° C.

The only experiments specifically designed to show the effects of light on virus concentrations in plants grown at different temperatures are those of Pound and Walker (6). They reported that photoperiods of 8 and 15 hours had no effect on the concentration of cabbage virus A in cabbage grown at 16° and 28°C, although symptom differences were noted. That light can affect the concentrations of cucumber mosaic virus (2) and of tobacco mosaic virus (4) in growing plants has been demonstrated recently. In addition, temperature studies (5) in which the light environment varied seasonally and light studies (4) in which temperatures were subjected to seasonal variation have indicated that light and temperature might interact in influencing virus concentration. These results indicate that cabbage virus A in cabbage might have been an unsuitable host-virus complex for a demonstration of light-temperature interactions. Consequently, it appeared that a re-examination of the problem would be of interest. The present investigation was undertaken under strictly controlled environmental conditions to ascertain whether or not light and temperature effects on virus concentration could interact. That is, would light effects be the same at different temperatures and would temperature effects remain unchanged under different light conditions? Preliminary observations showed that squash mosaic virus (SMV)[2] in squash and pumpkin would be a suitable host-virus complex to use. Relative SMV concentrations from leaf homogenates of systemically infected plants grown at various temperatures were first determined. The two extreme temperatures at which the virus multiplied well were chosen, and experiments in which photoperiod and temperature or light intensity and temperature were varied simultaneously were then performed.

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[2] The author wishes to thank Dr. G. S. Pound for the culture of squash mosaic virus.

MATERIALS AND METHODS.—Pumpkin (*Cucurbita pepo* L. var. Small Sugar) and Zucchini squash (*C. pepo* L. var. *Melopepo* Alef.) were grown in glazed earthenware crocks in soil. The crocks were placed in subterranean controlled-environment rooms, and the seedlings were grown 1 week under the various controlled conditions prior to inoculation of the cotyledons and primary leaves. In the temperature experiments, the light was left constant at 740 foot-candles for 20 hours a day, and the temperatures were varied at 15.5°, 21.0°, 26.5°, and 32.0°C. In the temperature-photoperiod experiments, plants were grown at 15.5° and 26.5°C under 740 foot candles at day lengths of 8 and 20 hours. In the temperature-light intensity experiments, the plants were grown at 15.5° and 26.5°C during 20-hour photoperiods under 220 and 740 foot-candles. The plants were harvested and weighed, after which the leaves were removed, weighed, and frozen. A sample consisted of all the leaves from not less than 4 plants.

The procedure used to obtain preparations for measurement on a Beckman Model DU spectrophotometer at 260 mμ was essentially the same as that of Rice et al (8). The frozen samples were homogenized in a Waring Blendor at a dilution of 1:11 (w/w) in deionized water. Portions were expressed through 2 layers of cheesecloth and centrifuged at approximately 2000 G for 15 minutes. The pH was then adjusted to 5.0 with 10 per cent (v/v) acetic acid, and the material was again centrifuged at 2000 G for 15 minutes. The clear amber samples were kept overnight at 4° C to allow host material to precipitate and were then centrifuged at 11,730 G (maximum) for 10 minutes in a Spinco Model L ultracentrifuge. The supernatants were decanted and centrifuged at 88,700 G (maximum) for 90 minutes. The supernatants were discarded, and each opaque colorless pellet was suspended in 6 ml of water. These infectious preparations were centrifuged at 11,730 G for 10 minutes and measured at 260 mμ. Equal numbers of virus-infected and of healthy plants were used for all preparations.

All results are expressed as mg SMV nucleoprotein/g wet weight of leaf tissue. The conversion of optical density to weight was made by weighing dried virus samples (24 hours at 105° C) of known optical densities on a Mettler microbalance type M5 (Table 1).

EXPERIMENTAL RESULTS. — *Air temperature.* — The incubation periods of SMV in pumpkin and squash during 20-hour photoperiods under 740 foot-candles were 4 days at 32.0° and 26.5° C, 6 days at 21.0°, and 8 days at 15.5°. The initial symptoms consisted of a mild mottling of the upper leaves. Mottling became more generalized and severe with time at 15.5° and 21.0° in both hosts. At 26.5°, masking was evident in pumpkin at about 30 days after inoculation; in squash, the interveinal tissue of the leaves was less extensive than normal at about 2 weeks after inoculation, and no reduction in symptom severity ensued. The plants grown at 32.0° became symptomless about 2 weeks after inoculation (Fig. 1). Generally, the symptoms in squash were more severe than those observed in pumpkin.

The optimum temperature for growth of diseased and healthy plants was between 21.0° and 26.5° C. Diseased plants weighed only slightly less than the controls.

In each experiment, the virus concentrations in leaf homogenates from systemically infected plants were usually measured at 3 different periods after inoculation. Assays made at 7 days after inoculation revealed that the virus multiplied initially more rapidly at 26.5° than at 15.5° C in both hosts. In 1 of 6 experiments in the photoperiod trials, higher virus concentrations were found at 26.5° than at 15.5° at 10 days after inoculation. Usually, however, it was found that by 10 days after inoculation the virus concentration in leaves of plants grown at 15.5° and 21.0° had increased to a level above the concentration found at higher temperatures (Table 2). The virus concentration of leaves from plants grown at 26.5° and 32.0° were always relatively low after the first assay. The data clearly indicated that low temperatures were ultimately more favorable to virus increase than were high temperatures. Although the assays were spaced too

TABLE 1.—*Dry weight of squash mosaic virus preparations differing in optical density*

Optical density at 260 mμ	mg/ml
3.173	0.469
1.589	0.229
1.158	0.159
0.798	0.112
0.581	0.084
0.419	0.060
0.281	0.039
0.144	0.020

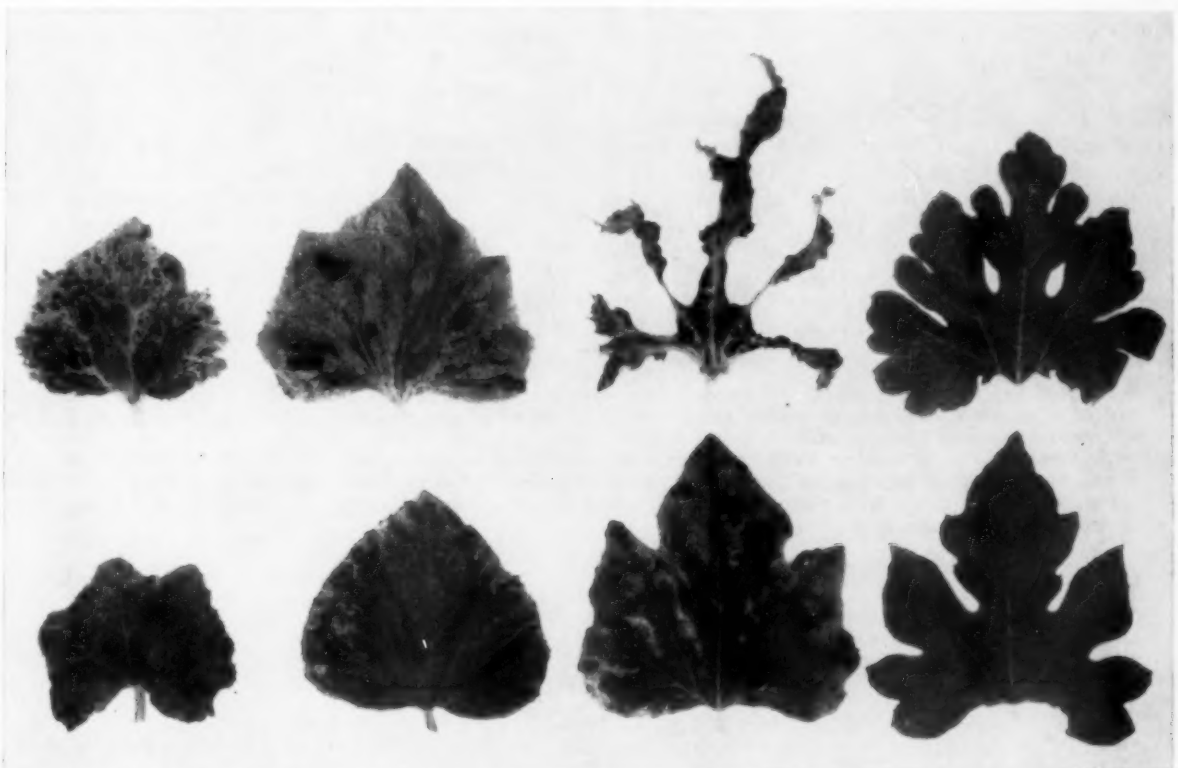

FIG. 1. Symptoms of infection by squash mosaic virus in squash (upper row) and in pumpkin (lower row) at 15.5°, 21.0°, 26.5°, and 32.0°C (from left to right) at 20 days after inoculation.

TABLE 2.—*Concentration of squash mosaic virus (SMV) in pumpkin and in squash plants grown at different air temperatures* [a]

Host and days after inoculation	Weight[b] of SMV (mg/g wet weight) in leaf tissue from plants grown at indicated temperature				Average wet weight (g) of leaves of infected plants grown at indicated temperature			
	15.5°C	21.0°C	26.5°C	32.0°C	15.5°C	21.0°C	26.5°C	32.0°C
Pumpkin								
10	0.20	0.27	0.16	0.08	3.9	5.2	8.6	5.5
20	0.16	0.18	0.07	0.06	8.7	12.2	12.6	10.7
30	0.09	0.10	0.08	0.05	12.0	15.0	16.9	16.3
Squash								
10	0.22	0.25	0.18	0.08	3.2	5.2	7.0	5.8
20	0.40	0.27	0.08	0.05	7.5	13.1	17.9	14.5
30	0.28	0.19	0.05	0.03	13.8	15.9	20.4	20.7

[a] Plants grown at 20-hour photoperiod of 740 foot-candles.
[b] Determined spectrophotometrically and converted to weight basis from a standard curve.

far apart to locate definitely the time of maximum virus concentration, it would appear that it occurred about 1 week after inoculation at the higher temperatures in both hosts and between 2 and 3 weeks in pumpkin and between 3 and 4 weeks in squash grown at the 2 lower temperatures.

Photoperiod and air temperature.—The short 8-hour photoperiods at 26.5°C did not reduce the incubation period of the virus. At 15.5°, however, symptoms usually appeared a day later in the 8-hour plants than in the 20-hour plants. The symptoms of the short-day plants grown at 15.5° never became as severe as did those of the long-day plants. Photoperiod did not affect symptom development at high temperatures. The short-day plants were chlorotic and stunted.

The virus concentration of leaf homogenates from plants grown during 8- and 20-hour photoperiods at 15.5° and 26.5°C was measured in 4 separate experiments with pumpkin as a host and in 2 separate experiments with squash. Assays made at 10 days after inoculation showed, with the 1 exception already noted, that the highest virus concentrations were always found in the low-temperature plants regardless of the photoperiod. At low temperatures, the photoperiod did not seem to have a marked effect on virus concentration at the 10-day assay; in 4 of 6 experiments, short-day plants had a slightly higher virus concentration than did long-day plants, and the opposite was true in 2 of 6 experiments. At high temperatures, in contrast, the short-day plants always had a lower virus concentration than did the corresponding long-day plants. The data obtained from the 20-day assays showed that a pronounced increase in virus concentration had occurred between 10 and 20 days after inoculation in the short-day plants grown at 15.5° (in 1 of 6 experiments the increase was not evident until the 30-day assay), whereas the concentrations in long-day plants grown at 15.5° were much lower than those in short-day plants but still higher than the concentrations found at either photoperiod at 26.5° C (Table 3). The virus concentration remained high in leaves of pumpkin grown during short photoperiods at 15.5° C for at least 40 days, as evidenced by 1 experiment, whereas it dropped considerably in squash in the interval between 20 and 30 days after inoculation. In contrast to the results obtained from plants grown at 15.5°, the virus concentrations from plants grown at 26.5° were not influenced to any marked extent by photoperiod at either the 20- or 30-day assays.

Light intensity and air temperature.—In these ex-

TABLE 3.—*Concentration of squash mosaic virus (SMV) in leaves of pumpkin and squash plants grown at different temperatures and photoperiods* [a]

Host and days after inoculation	Weight[b] of SMV (mg/g wet weight) in leaf tissue from plants grown at indicated temperature and photoperiod				Average wet weight (g) of leaves of infected plants grown at indicated temperature and photoperiod			
	15.5°C		26.5°C		15.5°C		26.5°C	
	8-hour	20-hour	8-hour	20-hour	8-hour	20-hour	8-hour	20-hour
Pumpkin								
10	0.21	0.21	0.09	0.14	1.2	2.4	2.1	6.9
20	0.41	0.15	0.06	0.05	0.8	12.0	3.5	22.7
30	0.41	0.14	0.05	0.06	5.6	20.2	9.5	29.5
Squash								
10	0.20	0.15	0.14	0.19	0.7	4.0	1.4	8.1
20	0.55	0.36	0.09	0.11	1.5	15.2	5.1	19.0
30	0.25	0.15	0.10	0.10	2.7	20.0	7.0	26.0

[a] Plants grown at light intensity of 740 foot-candles.
[b] Determined spectrophotometrically and converted to weight basis from a standard curve.

periments, symptom development was the same as that noted under the photoperiod section. The plants grown under low light intensities exhibited the same growth pattern as did those grown under short day lengths.

The initial assays made 7 days after inoculation in 4 experiments revealed that light intensity had no consistent effect on the virus concentration found in leaf homogenates from plants grown at 15.5° C. At 26.5°, however, the leaves of plants grown under 220 foot-candles had consistently lower virus concentrations than did those obtained from leaves of plants grown under 740 foot-candles. In both pumpkin and squash grown under 220 foot-candles for 20 hours a day at 15.5°, a marked increase in virus concentration occurred between the 7- and 18-day assays and between the 7- and 20-day assays, respectively. The increases found at low temperatures from leaf-assays of plants grown under 740 foot-candles were not as striking. In both pumpkin and squash, the virus concentration in leaves from the low-light low-temperature plants fell by a factor of approximately 2 in the interval between the second and third assays (Table 4). In the case of pumpkin, this result differed from that of the photoperiod studies. The concentration drop was also noted from assays made from the leaves of high light-intensity plants grown at the same temperature. The virus concentration of plants grown at high temperature showed a decrease rather than an increase during the period between the first and second assays. Light intensity had no marked effect on the virus concentrations, which remained quite steady during the interval between the second and third assays.

Discussion.—The temperature response of SMV in the leaves of growing squash and pumpkin plants was essentially the same as that reported for potato virus X in leaves of *Nicotiana glutinosa* L. and *N. tabacum* L. (5). The initial virus increase at high temperatures was not as great with potato virus X as it was with SMV, but both viruses reached greatest concentrations for the longest periods of time at temperatures of about 16° to 20° C. The demonstration that the virus content of the tip leaves may be different from that of all the leaves (1) obviates comparison of the results found with SMV with the tip-leaf assays performed with other predominantly cool-temperature viruses such as cabbage virus B in cabbage (6) and strains of turnip virus 1 in *N. glutinosa* (7). Nonetheless, it is interesting to note that most of the viruses studied up to the present time fall into a general category of viruses that reach greatest concentrations over long periods of time at cool temperatures, regardless of the type of tissue used for assay.

The generalization that changes produced in virus content by environmental agencies other than temperature are secondary to temperature changes (3) holds true when both light and temperature are varied. The temperature response of SMV remained essentially unchanged when light conditions were varied, whereas the response of the virus to light was markedly different at 15.5° and at 26.5°C. The finding that soon after inoculation long days and high light intensities favored virus multiplication at 26.5° agrees with those of other workers (2, 4, 9). Subsequent assays showed that this effect was not prolonged, whereas a striking virus increase occurred later in plants grown under poor light conditions at 15.5°, although no consistent early trend was noted. The response of SMV in squash and pumpkin to light was nonspecific in that essentially the same results were obtained with both photoperiod and light intensity. The tissue of plants grown under poor light conditions, particularly at 26.5°, was relatively fragile. When the difference in results obtained from plants grown under poor light conditions under the 2 temperatures is considered, it appears unlikely that the differences resulted from differential release of virus during extraction from leaves of plants grown under different conditions.

Although the time intervals between assays were too great to allow determination of the precise points at which virus content was highest or lowest, it is evident that a marked decrease in virus content did occur (after high virus concentrations were reached) within 30 days after inoculation in every type of ex-

Table 4.—*Concentration of squash mosaic virus (SMV) in leaves of pumpkin and squash plants grown at different light intensities*[a]

Host and days after inoculation	Weight of SMV (mg/g wet weight) in leaf tissue from plants grown at indicated temperature and light intensity				Average wet weight (g) of leaves of infected plants grown at indicated temperature and light intensity			
	15.5°C		26.5°C		15.5°C		26.5°C	
	220 foot-candles	740 foot-candles	220 foot-candles	740 foot-candles	220 foot-candles	740 foot-candles	220 foot-candles	740 foot-candles
Pumpkin								
7	0.04	0.07	0.11	0.17	0.5	2.0	0.8	5.8
18	0.47	0.25	0.13	0.09	1.7	10.5	2.5	16.2
30	0.26	0.16	0.13	0.10	3.4	17.1	4.0	15.0
Squash								
7	0.03	0.02	0.17	0.23	1.0	3.6	1.2	5.8
20	0.44	0.31	0.06	0.10	3.9	17.8	4.7	18.3
30	0.25	0.13	0.07	0.10	4.7	20.9	5.9	20.9

[a] Plants grown at 20-hour photoperiod.
[b] Determined spectrophotometrically and converted to weight basis from a standard curve.

periment except those performed with pumpkin at low temperatures under short photoperiods. That good growth of host and high concentrations of virus are not necessarily associated is illustrated by the high SMV concentrations found in the plants grown at 15.5° under poor light conditions. These plants were the weakest of any grown during the course of this work. The results of Welkie and Pound (10), in which manganese-deficient tobacco was found to support much higher levels of tobacco mosaic virus than did plants receiving an optimum level of the element, is a more striking illustration of this point.

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FACTORS INFLUENCING THE GERMINATION OF RESTING SPORANGIA OF PHYSODERMA MAYDIS [1]

T. T. Hebert and Arthur Kelman

SUMMARY

In an investigation of the influence of environmental factors on the germination of resting sporangia of *Physoderma maydis*, the following distinctive characteristics were observed: high minimum temperature, high optimum pH, and light requirement for germination. Cardinal temperatures for germination of resting sporangia were 18°, 28°, and 36°C. The optimum pH range for germination was pH 8.5 to 9.0; the minimum was near pH 3 and the maximum near pH 10. The sporangia failed to germinate in complete darkness. As the light intensity was increased from 0 to 12 foot-candles, germination percentages increased according to the logarithm of the intensity. Higher light intensities did not significantly increase germination. When exposed to light for 12 hours and then placed in darkness for 60 hours, sporangia germinated poorly; however, when sporangial suspensions were held for 24 hours in darkness and then exposed to light for 12 hours, good germination resulted. Highest germination was obtained under blue light, low germination under green light, and no germination under yellow or red light. Oxygen was found to be necessary for the light reaction and subsequent germination to occur. Sporangia germinated in the absence of carbon dioxide.

Brown spot of corn, caused by *Physoderma maydis* Miyabe (*Physoderma zeae-maydis* Shaw) is present every year to some extent in North Carolina. The severe damage sustained by susceptible hybrids in recent years has prompted the initiation of a program to develop hybrids with resistance to this disease. In the development of inoculation techniques for evaluating the resistance of corn breeding lines to brown spot (5), it was essential to standardize procedures involved in preparation of sporangial inoculum.

Factors affecting germination of resting sporangia of *P. maydis* have been studied previously by Tisdale (7) and by Voorhees (9). The former concluded that germination was controlled mainly by moisture, tem-

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perature, and aeration; however, he also noted that sporangia often failed to germinate, even when the above-mentioned factors were favorable for germination. In more extensive studies, Voorhees found that sporangia would not germinate in complete darkness or in direct sunlight but germinated well in diffuse sunlight or light from an electric lamp. Influence of light intensity and quality were not studied in detail. In distilled water adjusted to initial hydrogen-ion concentrations ranging from pH 2.1 to 8.1, highest percentages of germination were observed at pH 7.4. Voorhees also presented data showing that oxygen and carbon dioxide were necessary for germination. Critical determinations of the influence of temperature on germination were not made in previous investigations. The objective of the present study was to determine more precisely the influence of environmental factors on the germination of resting sporangia of *P. maydis*. Preliminary reports on certain phases of this study have been published (3, 4).

MATERIALS AND METHODS.—Resting sporangia of the brown spot fungus were obtained by grinding diseased portions of dried corn leaves in a Wiley Mill. This material was suspended in tap water or buffer solutions and passed through an 80-mesh screen to remove large particles and clumps of sporangia. For germination tests, 5 ml of a sporangial suspension containing approximately 50 sporangia per low-power field of the microscope were placed in 5-cm Petri dishes. The germination percentage was usually determined after 72 hours and was based on duplicate counts of 50 sporangia per dish, with 2–4 dishes per treatment.

In temperature studies, sporangial suspensions were placed in constant-temperature chambers and illuminated with 100 foot-candles of fluorescent light. For other experiments, unless otherwise indicated, sporangial suspensions were exposed to light from two 40-watt fluorescent tubes in a room maintained at 28± 2°C. Light intensity was measured with a Weston Model 756 light meter.

INFLUENCE OF TEMPERATURE ON GERMINATION.—Sporangial suspensions were exposed to a light intensity of 100 foot-candles in constant-temperature chambers adjusted to a range in temperature from 18° to 40°C. The results (based on a mean of 4 experiments) are presented in Figure 1,A.

At 18° and 40°C there was no germination at the end of 72 hours. After 144 hours at 18° only a very few sporangia had germinated, whereas none had germinated at 40° in this same period. Less than 1 per cent of the sporangia germinated at 36°. Highest germination occurred near 28°.

Under field conditions the sporangia are exposed neither to continuous light nor to constant temperatures. Therefore, an experiment was designed to determine the effect of alternating a period of 12 hours in darkness at one of 6 different temperatures with a period of 12 hours in light at 28°C. During the period of incubation in darkness, sporangia were held at 8°, 12°, 16°, 20°, 24°, and 28°C.. When readings were taken after 48 hours, the germination percentages were correlated with the dark-period temperature, with lowest germination at lowest dark-period temperature (Fig. 1,B). After 72 hours, however, a relatively high percentage of sporangia (62–74 per cent) had germinated at all temperatures.

GERMINATION OF SPORANGIA IN RELATION TO HYDROGEN ION CONCENTRATION.—In the evaluation of the influence of hydrogen ion concentration on the germination of sporangia, 0.01 M to 0.02 M solutions of potassium phosphate buffer were adjusted to a range from pH 3.3 to pH 10.4 by addition of 0.1 N HCl or KOH. Sporangia were added to 5-ml portions of the buffered solution at each pH level. The highest percentage of sporangia germinated at the highest pH level used; however, the pH at this level had decreased from pH 10.4 to pH 8.2 in 72 hours.

To minimize the change in pH in the more alkaline solutions, sporangia were germinated in 100 ml of buffer in 250-ml beakers. The pH at each level was

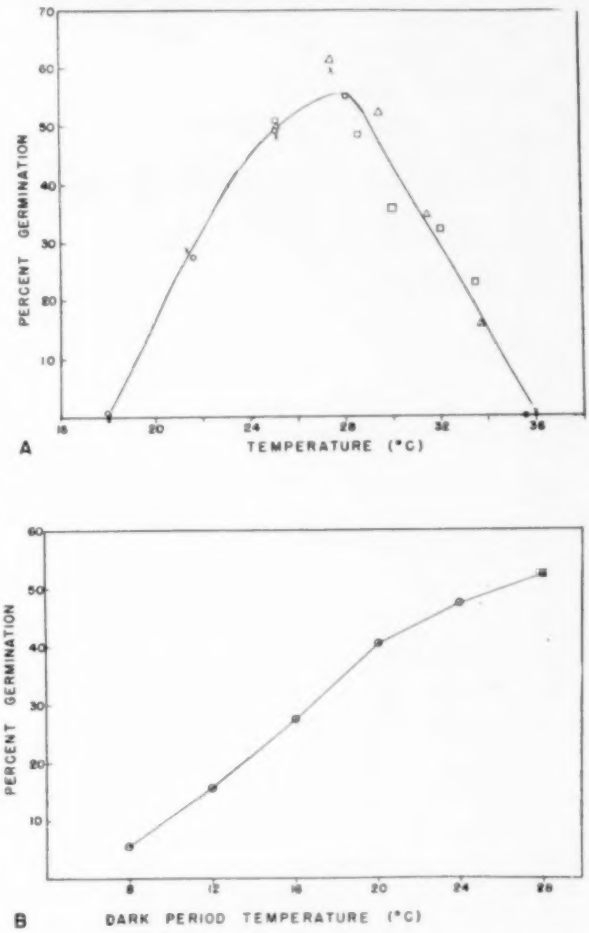
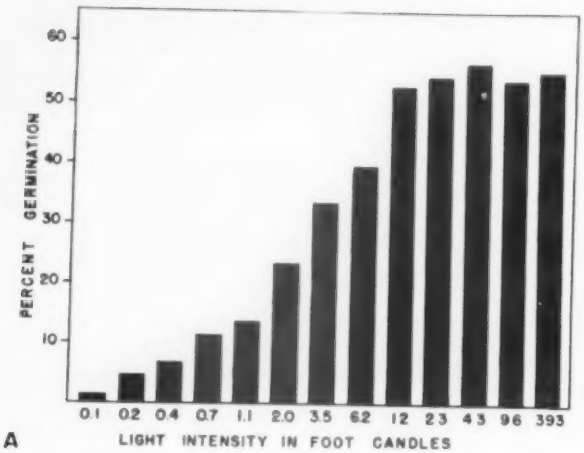


FIG. 1. Effect of temperature on germination of resting sporangia of *Physoderma maydis*. A) Constant temperature and continuous light (each of 4 tests represented by different symbols). B) Alternating periods of 12 hours at 28°C in light with 12-hour periods of darkness at given temperatures.



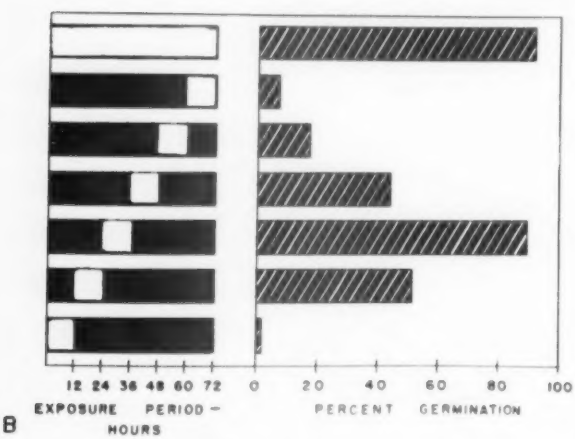


FIG. 2. Effect of light intensity and period of exposure on germination of resting sporangia of *Physoderma maydis*. A) Percentage germination at stated light intensities. B) Germination induced by 12-hour light exposure periods (white spaces in bars) at given intervals of time following suspension of sporangia in water. Germination in continuous light (top bar) included for comparison.

readjusted if necessary at the end of 24 and 48 hours.

The minimum pH for germination appears to be near pH 3, the optimum between pH 8.5 and 9, and the maximum near pH 10 (Table 1). Because of the difficulty of maintaining pH at constant levels during the period of incubation, it was not possible to make a precise determination of these points.

EFFECT OF LIGHT ON GERMINATION.—In preliminary experiments it was found that sporangia failed to germinate in complete darkness but germinated in diffuse light, thus confirming the results of Voorhees (9). An investigation was then made of the effect of light intensity, light quality, and length of exposure period to light on sporangial germination.

Light intensity.—Sporangia were germinated at a series of light intensities ranging from 0 to 393 foot-candles. Light intensity was varied by interposing a varying number of sheets of white paper between the light source and the sporangial suspensions. Mean germination percentages for 2 experiments are presented in Figure 2, A.

A light intensity as low as 0.1 foot-candles produced a measurable response in germination. The percentage germination increased rapidly as light intensity was increased up to 12 foot-candles. When the percentage germination for light intensities from 0.1 to 12.0 foot-candles was plotted on logarithmic probability paper, a straight line was obtained, showing that the response in germination was proportional to the logarithm of the light intensity. When light intensity was increased from 12 to 393 foot-candles, no significant increase in germination percentages resulted.

Period of exposure to light.—In the above experiments, the sporangia were maintained at the indicated intensities under continuous illumination. Since sporangia of *P. maydis* do not receive continuous illumination in nature, experiments were initiated to determine the effect of intermittent periods of exposure to light on germination. Results of the first of these experiments indicated that germination was as high with alternating 12-hour periods of darkness and light as with continuous illumination (26 foot-candles). Sporangia were then exposed for single 12-hour periods beginning at 0, 12, 24, 36, 48, and 60 hours after suspension of the sporangia in water. Maximum germination resulted when the light exposure occurred between the 24th and 36th hour after the sporangia were suspended in water, and only a small percentage of the sporangia germinated when exposed to light during the first 12 hours after suspension (Fig. 2, B). The low level of germination that occurred when the light-exposure period was delayed until the 48th and 60th hour after suspension was probably caused partly by the toxic action of metabolites of other organisms growing in the suspension. An increase in germination percentage was obtained by resuspension of the sporangia in fresh water before the exposure to light.

An experiment was completed to determine the

TABLE 1.—*Germination of sporangia of Physoderma maydis at different pH levels*

pH		Percentage germination after 72 hours
Initial	Final	
2.9	3.3	0
4.0	4.2	6
5.0	5.1	23
6.0	6.0	41
7.1	7.0	45
8.1	7.9	61
9.1	8.5	85
10.1	9.3	62[a]
11.0	10.3	0
11.4	10.6	0

[a] A high percentage of the lids in the walls of the sporangia had opened partially at this pH, but only a few formed zoospores.

effect on germination of short intermittent light-exposure periods. Sporangia were suspended in water, maintained in darkness for the first day, and exposed to light for different periods of time during the second and third days. The lengths of the light exposure periods on the second and third days were as follows: 1) 8 hours; 2) 4 hours; 3) 30 minutes once an hour for 8 hours; and 4) 15 minutes once an hour for 8 hours. The germination percentages for the above 4 series were as follows: 1) 95, 2) 91, 3) 91, and 4) 87. The results indicate that long periods of continuous light are not necessary for high germination of sporangia.

Light quality.—A comparative study was made of the effectiveness of various wave lengths of light in inducing germination. Sporangial suspensions were placed under the following Corning glass filters: Signal Red, Lantern Yellow, Sextant Green, Lantern Blue, and Lunar White. The mean germination percentages for 2 experiments were 0 in red or yellow light, 15 in green light, 49 in blue light, and 46 in white light.

Although sporangia in each of the above experiments were exposed to approximately the same number of foot-candles of each wave length, the sporangia exposed to blue light received more energy than those exposed to yellow light, since the energy of light is inversely proportional to its wave length. To determine whether the difference in effectiveness of blue and yellow light in inducing germination is due to their difference in energy levels, sporangia were exposed to 3 foot-candles of blue light and 72 foot-candles of yellow light. Germination percentages were 61 in blue light and 0 in yellow light, indicating that the difference in germination levels in blue and yellow light results from differences in the effect of light quality rather than to different energy levels.

EFFECT OF OXYGEN AND CARBON DIOXIDE.—Certain photochemical reactions in higher plants occur only in the presence of oxygen (1). To determine whether oxygen is necessary for the response to light of the sporangia of *P. maydis*, sporangia were germinated in an oxygen-free atmosphere obtained by passage of air through a solution of pyrogallol and potassium hydroxide. The treatment combinations and germination percentages are presented in Table 2.

Sporangia did not germinate when exposed to light in the absence of oxygen. Suspension of the sporangia in water in the presence of oxygen prior to light exposure also appeared to be necessary for good germination.

The alkaline pyrogallol solution used to remove oxygen from the air in these experiments also removed carbon dioxide. Voorhees (9) reported that carbon dioxide was necessary for germination of sporangia of *P. maydis*. Therefore, an experiment was made to test the germination of sporangia in air from which carbon dioxide had been removed by passage through a potassium hydroxide solution. It was found that removal of carbon dioxide had no effect on sporangial germination.

The experiment was repeated with the corn smut fungus, *Ustilago maydis* (DC.) Cda., as an internal check on the removal of carbon dioxide. It has been shown that only a very low percentage of the spores of this fungus will germinate in the absence of carbon dioxide (6). In this test, 21 per cent of the sporangia of *P. maydis* germinated in the presence of carbon dioxide and 24 per cent in the absence of carbon dioxide. Under the same conditions, 44 per cent of the chlamydospores of the smut fungus germinated in the presence of carbon dioxide, but less than 1 per cent germinated in the absence of carbon dioxide. Similar results were obtained when barium hydroxide was used to remove the carbon dioxide. These results are not in agreement with those of Voorhees (9), who reported that the sporangia of *P. maydis* would not germinate following removal of carbon dioxide with barium hydroxide.

DISCUSSION.—The study of factors affecting germination revealed that *P. maydis* is distinctive among the fungi in a number of respects. The minimum temperature of 18°C required for germination of resting sporangia of *P. maydis* appears to be higher than that necessary for spore germination of most fungi (8, 10). In a survey of the literature on spore germination Gottlieb (2) noted that only 2 of 80 fungus species reviewed required a minimum temperature greater than 10°C for germination of spores. This high minimum temperature for germination may be an impor-

TABLE 2.—*Germination of sporangia of Physoderma maydis when exposed to light in the presence and in the absence of oxygen*

Exposure periods[a]				Percentage germination	
1 0-24 hours	2 24-36 hours	3 36-48 hours	4 48-72 hours	Test 1	Test 2
Dark, − 0	Light, + 0	Dark, + 0	Dark, + 0	0	0.5
Dark, + 0	Light, + 0	Dark, − 0	Dark, + 0	43	30
Dark, + 0	Light, − 0	Dark, + 0	Dark, + 0	[b]	0
Dark, + 0	Dark, + 0	Light, + 0	Dark, + 0	46	16
Dark, + 0	Dark, + 0	Light, − 0	Dark, + 0	0	0
Dark, + 0	Dark, − 0	Light, + 0	Dark, + 0	27	11
Dark, − 0	Dark, + 0	Dark, + 0	Light, + 0	[b]	36
Dark, + 0	Dark, + 0	Dark, + 0	Light, + 0	[b]	27

[a] + 0 = presence of oxygen; − 0 = absence of oxygen.
[b] Treatment not included.

tant factor in the epiphytology of the disease, since field symptoms usually appear after periods of warm weather in late spring or early summer.

The optimum pH for germination was found to be higher than the optimum of pH 7.4 reported by Voorhees (9); however, Vorhees used distilled water instead of buffer solutions in his experiments. No reports were found in the literature of fungi with an optimum pH for spore germination above pH 8. The significance of this finding in the epiphytology has not been determined. It seems unlikely that the water that collects in the whorl of corn plants under field conditions has an alkaline reaction.

Perhaps the most interesting characteristic of sporangia of *P. maydis* is their requirement of light for germination. No reports that light is essential for germination of spores of other fungi were found in the literature.

The germination of sporangia of *P. maydis* and the phototropic responses of certain fungi and of etiolated higher plants appear to have similar action spectra. The photoreceptor for phototropic responses in higher plants is believed by some investigators to be riboflavin or perhaps a yellow enzyme, whereas others suggest that carotenoids are the likely photoreceptors (1). Chemical analyses were made to determine whether riboflavin or carotenoids are present in the sporangia. The sporangia proved to be relatively rich in riboflavin, containing 8.4 μg/g compared to 5.0 for healthy corn leaf tissue and 4.6 for leaf tissue surrounding brown spot lesions. In contrast, the concentration of carotenoid pigments in sporangia was considerably lower than that of normal corn leaf tissue.

Galston (1) has postulated that the phototropic responses of etiolated plants are due to the photoinactivation of 3-indoleacetic acid by riboflavin. In an effort to determine whether a similar reaction occurs in the sporangia of *P. maydis*, these sporangia were germinated in aqueous solutions of riboflavin, indoleacetic acid, indolebutyric acid, and napthaleneacetic acid at concentrations ranging from 0.001 to 10 ppm in a low light intensity. There was no indication from these tests that these chemicals had any influence on the light requirement for germination.

P. maydis possesses several advantages for studies on the mechanism of light action. The sporangia are single celled and can be collected from diseased corn plants and maintained in large quantities. The germination response to light can be determined easily and accurately. Space required for germination tests is small, and environmental conditions under which the experiments are conducted can be easily controlled.

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Reactions of Some Alfalfa Varieties to the Stem Nematode.[1] OLIVER F. SMITH.[2] The stem nematode, *Ditylenchus dipsaci* (Kühn) Filip., is a serious pest of alfalfa in many of the irrigated valleys of the Pacific coast and intermountain areas of the West.[3] Damage to alfalfa by this eelworm varies from year to year, but it always curtails production of hay. The most obvious effect of light infection is the production of fewer stems than normal, whereas severe infection often causes death of the plants. Injury is most pronounced in early spring, as moisture and slow growth of the plants favor infection.

Nematode injury is located principally in the crown, affecting the young buds and stem bases.[4] Infected buds become thickened and deformed and usually do not elongate into stems. Infected plants make very little early spring growth, and field plots of susceptible varieties are easily detected in the spring of the year when nematode infection is severe.

Since many of the recently released varieties of alfalfa are well adapted to climatic conditions of the West where the stem nematode is present, it seemed advisable to test them for resistance to this eelworm. Accordingly, tests were made in field row plots where natural infection by the nematode is usually severe.

The varieties tested were seeded in duplicate field row plots on April 24, 1955. There were approximately 200 plants per row. Nematode-infected alfalfa buds were scattered over the plots in August of that year to hasten infection. The plots were irrigated frequently to encourage development and spread of the nematode. Infection was quite evident in susceptible varieties by late October of 1955 and was severe by April, 1956. The resistant variety Lahontan and Nevada Synthetic E showed vigorous spring growth with no injury from the nematode, whereas nematode-susceptible varieties such as Ladak, Vernal, and Ranger showed very little or no spring growth, because of injury by the stem nematode (Fig. 1).

The estimated percentage of plants infected in the various varieties is recorded in Table 1. Infection was as low as 2 per cent in the resistant variety Lahontan, whereas highly susceptible varieties such as Ladak were completely infected. Of the synthetics and varieties tested, plant infection by the nematode was less than 10 per cent in Lahontan, Nemastan, and Nevada Synthetic E, approximately 50 per cent in Talent, and over 90 per cent in all other varieties. Alfalfas showing less than 10 per cent infection are considered highly resistant to the stem nematode.

TABLE 1.—*Estimated percentage of plants infected by the stem nematode in field row plots of 15 alfalfa varieties and 1 synthetic at Reno, Nevada*[a]

Syn. or variety	Percentage of plants infected	Syn. or variety	Percentage of plants infected
Atlantic	100	Nemastan	7
Buffalo	96	Nevada Syn. E	8
Caliverde	100	Orestan	97
DuPuits	94	Ranger	97
Grimm	100	Rhizoma	100
Ladak	100	Sevelra	100
Lahontan	2	Talent	50
Nomad	100	Vernal	100

[a] Plots were seeded May 24, 1955, and notes on infection were recorded April 14, 1956.

The fact that all plants of some varieties were not infected does not necessarily mean there are plants within those varieties that are highly resistant to the stem nematode. The plants that were not infected may have been escapes. Their resistance has not been rechecked to determine that possibility.—Agricultural Experiment Station, University of Nevada, Reno, Nevada.

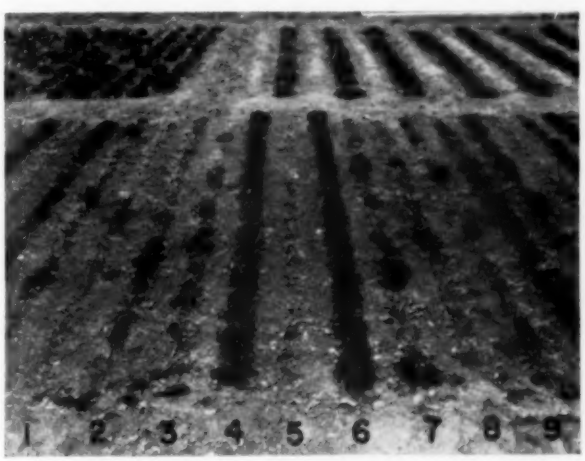

FIG. 1. Single-row plots of alfalfa varieties showing relative damage by the stem nematode. Susceptible varieties show very little or no spring growth because of damage by the stem nematode, whereas resistant varieties show healthy, vigorous spring growth. 1) Nomad. 2) Sevelra. 3) Ranger. 4) Nevada Synthetic E. 5) Ladak. 6) Lahontan. 7) Orestan. 8) Grimm. 9) Vernal. Plots seeded May 24, 1955; photographed April 14, 1956.

[1] Cooperative investigations by the Nevada Agricultural Experiment Station and the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

[2] Research Agronomist, Crops Research Division, ARS, U. S. Department of Agriculture.

[3] Thorne, G. 1932. Alfalfa-stem nematode causing severe damage in some western areas. Yearbook Agr. (U. S. Dept. Agr.) 1932: 99-101.

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Itersonilia perplexans on Sunflowers in Uruguay.[1] W. E. Sackston. Sunflower seedlings of the variety La Estanzuela were inoculated in the greenhouse at La Estanzuela with *Puccinia helianthi* Schw., the cause of sunflower rust, by exposing them to sporidial discharge from leaves bearing telia. Five days after inoculation, the cotyledons and leaves of some seedlings were severely lesioned by an organism other than the rust pathogen; 7 days after inoculation, symptoms were severe on most of the plants exposed. The lesions varied from isolated circular brown spots, 2–4 mm in diameter, to larger irregular areas, up to 10 mm long and 5 mm wide. Lesions caused by coalescence of individual smaller spots involved leaf areas up to 15–20 mm long and 5–10 mm wide. Several cotyledons were dead, and others were beginning to shrivel as smaller lesions coalesced to form large necrotic areas. The symptoms are illustrated in Figure 1.A.

Lesioned leaves were surface sterilized and plated. The plates were inverted during incubation. Within 2 days, white spore deposits were observed on the Petri dish covers under the lesioned leaf tissues (Fig. 1.B). The spores were the typical lunate ballistospores of *Itersonilia* sp., apparently *I. perplexans* Derx,[2, 3] a member of the Heterobasidiomycetes, first reported as a pathogen of sunflowers in Manitoba, Canada.[4] When sunflower leaves of the same sample that was used to provide the telial inoculum in the greenhouse were suspended over potato-sucrose agar in Petri dishes, numerous colonies of *I. perplexans* developed.

Seedling sunflower plants of various ages were inoculated in the greenhouse to test the pathogenicity of the cultures isolated. Some plants were inoculated by suspending over them plated lesioned leaves out of which colonies of *I. perplexans* were growing. Others were exposed to cultures of the fungus on agar in Petri dishes. Still other plants were exposed to spore discharge from rusted leaves collected in field plots for telial inoculations. Within 5 days, typical lesions appeared on most leaves of the inoculated plants. Plants exposed to rusted leaves from the field showed symptoms earliest and developed most lesions. Non-inoculated check plants remained healthy.

In September, 1956, volunteer seedlings in a plot at La Estanzuela were observed to have numerous lesions on the cotyledons and first true leaves. A few individual lesions, 2–4 mm in diameter, were present, but on most leaves the lesions had coalesced to cover from one-quarter to one-half the surface, killing many cotyledons and a few of the true leaves.

Lesioned cotyledons and leaves were plated on agar, and the plates were inverted. Some colonies of *I. perplexans*, free of contaminants, grew out from the lesions; others were overrun by various fungi. Heavy deposits of typical ballistospores of *I. perplexans* formed on the Petri dish covers under the lesioned material. Inoculations in the greenhouse proved the pathogenicity of the fungus, which was reisolated in pure culture from the experimentally induced lesions on test plants.

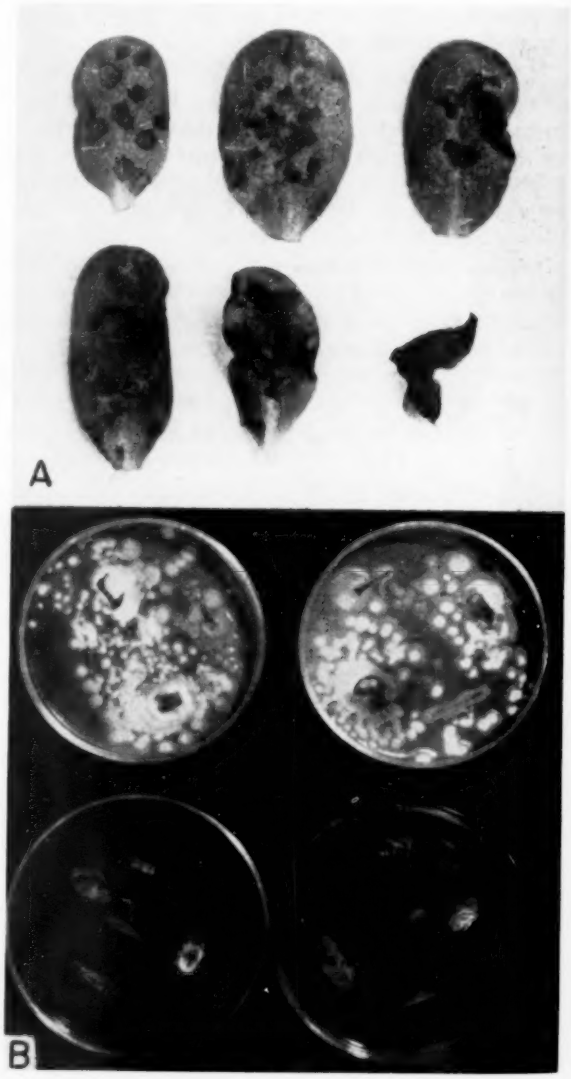

Fig. 1. A) Sunflower cotyledons lesioned by *Itersonilia perplexans* Derx in the greenhouse at La Estanzuela, Colonia, Uruguay. B) Platings of tissues from sunflower seedlings. Upper row: left, exposed to spore discharge from leaves with telia, collected in the field; right, exposed to spore discharge from colonies of *I. perplexans*. Lower row: deposits of ballistospores of *I. perplexans* on covers of Petri dishes that were inverted during incubation.

[1] Contribution No. 1631 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario. Work done while the author was on loan from the government of Canada to the government of Uruguay.

[2] Derx, H. G. 1948. Itersonilia, nouveau genre de sporobolomycètes à mycélium bouclé. Bull. Botan. Gardens, Buitenzorg. Ser. 3. 17:465-472.

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Lesions caused by *I. perplexans* have not been found previously on sunflower plants in the field. The fungus has not been recorded previously from Uruguay nor has any report been seen of its occurrence in other countries of South America.—Plant Pathology Laboratory, Science Service, Winnipeg, Manitoba.

LISTING OF BOOKS

PHYTOPATHOLOGY will, from time to time as space becomes available, list titles of books on pathological subjects. A brief description of the contents of the books will be included.

STAKMAN, E. C., AND J. G. HARRAR, 1957. **Principles of plant pathology.** The Ronald Press Co., New York. 581 p. Price $8.00.

This book was designed for students specializing in plant pathology and, as a reference, for scientists concerned with the problem of increasing the world's food production. The authors develop and illustrate the fundamental principles and concepts of plant pathology as they have seen them validated by observation, experimentation, and research. The book deals with the nature of classification of plant pathogens, the ecologic and physiologic relationships between host and pathogen, the nature and development of epidemics, and the international aspects of plant disease control. Emphasis is on the pathogens that attack food-producing plants. The appendix contains a list of important books on plant pathology and an alphabetical listing of the principal pathogens cited in the text.

WESTCOTT, CYNTHIA. 1957. **Plant doctoring is fun.** C. Van Nostrand Co., Inc., Princeton, New Jersey. 280 p. Price $4.50.

This is Cynthia Westcott's own story of why and how she became a plant doctor and of her varied experiences with people and with plant pests before and after she registered her trade name, The Plant Doctor. She writes of her childhood, of graduate study at Cornell, of collecting and camping trips, of visiting and treating gardens, of lecturing and writing, of attending conventions, and of working and experimenting in her back yard. Miss Westcott emphasizes the fun she has had in learning how to recognize diseases and pests and in teaching others about them.

REPORT OF THE 49TH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The American Phytopathological Society held its 49th Annual Meeting in conjunction with the American Institute of Biological Sciences at Stanford University, Stanford, California, on August 26–28, 1957. The Council met on August 25, 26, and 28, and general business meetings of the Society were held on August 26 and 28. There were 167 contributed papers of original research presented by members. These were divided into sections on Virology, Diseases of Cereal and Field Crops, Diseases of Vegetable Crops, Physiology of Diseased Plants, Diseases of Fruit Crops, Virus Diseases of Fruits, Virus Diseases of Cereal, Field and Vegetable Crops, Nematology, Diseases of Ornamentals and Forest Trees, Seed and Soil Treatments and Fungicides, Mycology and Physiology of Fungi, and Fungicides. The Society co-sponsored a symposium, Biology of Nematodes Attacking Horticultural Crops, with the American Society for Horticultural Sciences. The full-day program on August 29 and the Annual Meeting of Joint Committee on Grassland Farming also were co-sponsored by the American Phytopathological Society and the American Society of Plant Physiologists.

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REPORTS OF OFFICERS, COMMITTEES, AND REPRESENTATIVES

Report of the President. A brief summary of A.P.S. questionnaire on national conventions follows:

1) Apparently it is the opinion of a very substantial majority of the membership that our National Conventions have become too congested. The same majority believe that something should be done about the situation. The following means have found favor:
   a) Reduce the limit from 2 to 1 paper per members, including sole, senior, or junior authorship.
   b) Screen out low-quality papers. This is considered to be a responsibility of the Department Head or similar administrative officer rather than of a "screening committee" selected from the membership itself.
   c) Both *a* and *b* above. (This was the top choice in the returns from the questionnaire.)
   d) Encourage more presentation of papers at Divisional Meetings.
   e) Fewer papers per session, with more time for questions and discussion.
   f) "Educate" administrators that the practice of making reimbursement, whether in full or in part, contingent on the presentation of a paper or two may well influence the quantity and perhaps occasionally the quality, of the papers presented.
   g) Reduce the present 15-minute maximum presentation time to 10 minutes.
2) Time should be called strictly on speakers, with the chairman of the session being ruthless if the speaker is careless enough or thoughtless enough to force him to do so.
3) Rehearsal of papers before a critical "home" audience is strongly recommended; this should be sufficiently in advance of the convention to enable speakers to take advantage of constructive criticisms. Such a "dress rehearsal" would not only improve the presentation at the meeting itself but also contribute toward the speaker's staying within his allotted time.
4) A substantial majority of the membership believe that, except for the annual banquet, the evenings should be left open.
5) Considerable desire was expressed for more symposia, in the evenings if necessary, but not for more paper sessions in the evenings. The symposia, staged by recognized authorities in the various fields, are considered to be an efficient means of helping the membership keep abreast of more than just their own particular little specialties.
6) There is considerable room for improvement in the quality of the papers presented at our National Conventions. The opinion expressed by the membership would indicate that they consider less than half of the papers are good. "Quality" is deemed to include both subject matter and presentation and should be a matter of pride with the individual and a joint responsibility with his immediate administrative superior.
7) There is overwhelming opinion that we should continue our present tradition of the annual dinner and program. Most felt that $5 is a little too costly and that the upper limit should be around $4.
8) A great majority preferred a combination of levity and seriousness in the program at the annual dinner but are quite willing to leave it up to the local committee to determine the program.
9) The advantages of and the purposes served by our National Conventions, in order of importance as rated by the membership, are as follows: exchange of information by attending and participating in paper sessions; opportunity for personal conferences of a professional nature; visit with old friends and renew acquaintances; opportunity for graduate students and the younger members in general to meet and become acquainted with the older members; and job contacts (interviews between prospective employer and employee, to get leads on jobs, on men to fill jobs, etc.).
10) Opinion is fairly well divided on the comparative value of meeting with the A.I.B.S., alone, or with the A.A.A.S., with the preference indicated in that order. The same is true with regard to location, except that a preference was shown for college and university campuses over hotels and resorts, although these locations did receive a significant number of votes.

GEORGE W. FISCHER, *President*

Report of the Treasurer.

Receipts:		
Balance on hand July 1, 1956	$ 4,513.06	
Membership dues	9,957.00	
Sales of Fungicide Data Summaries	432.75	
Rockefeller Foundation grant for 50th Anniversary	9,000.00	
Total receipts		$23,902.81
Expenditures:		
Office of President	$ 235.97	
Office of Secretary	1,076.90	
Office of Treasurer	184.19	
Expenses, Annual Meeting (Cincinnati)	858.52	
Expenses, Fungicide Data Summaries	668.50	
Expenses, Public Relations Committee	355.00	
Expenses, 50th Anniversary Committee	204.97	
Expenses, Placement Committee	9.99	
Reserve for 50th Anniversary Celebration	9,000.00	
Engraved plate for British Mycological Institute	18.00	
Contribution to Joint Committee on Grasslands Farming	10.00	
Refund	3.00	
Total expenditures		12,625.04
Balance on hand, June 30, 1957		$11,277.77

SAUL RICH, *Treasurer*

Report of the Secretary. The American Phytopathological Society had 1798 members on August 6, 1957. This includes 113 Life Members, 17 Emeritus Members, and 13 Sustaining Associates. This year, to August 6, there have been 107 New Member Applicants of which 38 are students. Since the 1956 meeting, word has been received in the Secretary's office of the death of the following members and former members: E. A. Bessey, Charter Member; D. C. Buckland, C. E. F. Guterman, E. P. Meinecke, George T. Moore, C. E. Owens, Robert D. Shealy.

WM. B. HEWITT, *Secretary*

Report of the Business Manager of *Phytopathology*.

Receipts:

Balance on hand, July 1, 1956	$24,170.07	
Subscriptions and member-subscriptions	19,778.20	
Advertisements	3,382.75	
Sales of back issues	1,286.10	
Reprints and excess illustrations	5,213.56	
Sustaining Associate memberships	1,300.00	
Rockefeller Institute grant to PHYTOPATHOLOGY	600.00	
Interest on investments	1,091.73	
Total receipts		$56,822.41

Expenditures:

Manufacture and distribution of PHYTOPATHOLOGY	$20,206.85	
Cost of reprints	1,701.29	
Office of Business Manager	1,198.64	
Office of Editor-in-Chief	4,504.40	
Office of Advertising Manager	467.95	
Cost of new investments	195.77	
Collector of Internal Revenue	1,604.52	
Newsletter	36.19	
Purchase of back issues	40.00	
Refunds	18.25	
Total expenditures		30,303.86
Balance on hand, June 30, 1957		$26,518.55

SAUL RICH, *Business Manager*

Invested Funds of the American Phytopathological Society and *Phytopathology*. Amounts as of June 30, 1956. Dividends and interest for fiscal year, July 1, 1956, to June 30, 1957.

Sinking Fund:

U. S. Savings Bonds, Series J (at maturity)	$ 2,500.00
U. S. Savings Bonds, Series F (at maturity)	425.00
Northwestern Fed. Sav. & Loan Assn. (certificate) (Div. 70.00)	2,000.00
Family Finance Corp. common, 7 sh at par (Div. 10.48)	7.00
Westinghouse Electric Corp., 22 sh at cost (Div. 44.00)	849.08
Standard Oil of Ohio, common, 60 sh at cost (Div. 150.00)	2,018.65
American Cyanamid Co., common, 40 sh at cost (Div. 115.00)	2,160.47
Johns-Manville Corp., common, 57 sh at cost (Div. 125.44)	1,946.90
New Haven Savings Bank, on deposit (Int. 6.17)	204.05
Total	$12,111.15

Lyman Memorial Fund:

Westinghouse Electric Co., common, 35 sh at cost (Div. 70.00)	$ 1,350.81
American Tel. & Tel., common, 20 sh at cost (Div. 171.00)	2,997.47
Total	$ 4,348.28

Phytopathology Contingency Fund:

Johns-Manville Corp., common, 14 sh at cost (Div. 30.81)	$ 466.00
Arlington and Fairfax Sav. & Loan Assn. (Int. 241.23)	7,363.25
Prudential Bldg. Assn. (Int. 226.44)	6,413.57
Arundel Fed. Sav. & Loan Assn. (Int. 318.21)	8,407.12
Southern Fed. Sav. & Loan Assn. (Int. 361.80)	8,955.33
Decatur Fed. Sav. & Loan Assn. (Int. 286.24)	8,107.74
Fidelity Fed. Sav. & Loan Assn. (Int. 304.43)	7,535.42
Southwest Sav. & Loan Assn. (Int. 297.88)	7,373.95
Lawn Sav. & Loan Assn. (certificate) (Int. 280.00)	8,000.00
Total	$62,622.38

50th Anniversary Fund:

Standard Fed. Sav. & Loan Assn. (Int. 261.60)	$ 9000.00

SAUL RICH, *Treasurer and Business Manager*

Report of the Auditing Committee. On July 17, 1957, an audit was made of the financial records of The American Phytopathological Society and of PHYTOPATHOLOGY for the period July 1, 1956, to June 30, 1957. All receipts, expenditures and funds were found to be duly and accurately recorded and in order.

JAMES G. HORSFALL
PAUL E. WAGGONER
A. E. DIMOND

Report of the Editor-in-Chief. Volume 47 will be the largest single volume of PHYTOPATHOLOGY to date. The largest previous volume, Volume 24, contained 1386 pages, which is equivalent to about 730 pages of the present format. Volume 47 will contain approximately 754 pages.

Since the beginning of the year, 4 research papers have been accepted under the new rule permitting the publication of papers by nonmember authors from subscribing institutions in countries other than North American. Three of these will be published in Volume 47; the fourth will be in Volume 48, since it is the second received from 1 institution. These manuscripts were submitted from Kenya, Southern Rhodesia, and Ceylon.

Volume 47 contains the first modern-process color photograph to be published in PHYTOPATHOLOGY. This was not entirely successful, since gremlins invaded the printing press and left their bright-orange footprints on the prints in some copies. Our printer, Mr. John Ferguson, 3rd, was worried more by this than was anyone else, and he insisted that the matter be corrected. So that subscribers who received such prints can replace them with perfect ones, another print of this photograph will be bound in each copy of the August issue.

The first 10 numbers of Volume 47 will contain 634 pages of technical material, 7.5 pages of news, 56.5 pages of advertisements, and two 1-page drawing guides. The technical material consists of 106 articles, 2 unsolicited review articles, 29 Phytopathological Notes, 5 symposium papers, 3 obituaries, the report and 250 abstracts for the 48th Annual Meeting, 158 abstracts for the 49th Annual Meeting, the report and 14 abstracts for the 1956 Annual Meeting of the Northeastern Division, the report and 14 abstracts for the 1957 Annual Meeting of the Southern Division, the report and 20 abstracts for the 1957 Annual Meeting of the Potomac Division, 11 book listings, and 1 notice to authors.

On July 26, page proof for the August issue was on hand, the September and October issues were ready for the printing of page proof, and 69 manuscripts were in the process of being edited. Of these, 4 were in the hands of reviewers, 1 was being edited in this office, 30 had been returned to authors for revision, 30 were at the printer's, and 4 were in galley proof. From November 13, 1956, to July 26, 1957, 9 manuscripts had been rejected by the editors or withdrawn by the authors.

During the winter months, our printer became overloaded with unexpected work and fell behind schedule in the

printing of PHYTOPATHOLOGY. The situation has been corrected, and publication of papers in PHYTOPATHOLOGY is now about as prompt as is possible. For the August issue, the average time between receipt and scheduled publication was 190 days—50 days from receipt to acceptance and 140 days from acceptance to publication. Of the 50 days before acceptance, 14 were required for review by the editors, 31 for revision by the authors, and 5 for handling and editing in this office. Of the 190 days after acceptance, 40 were required for printing of galley proofs, 6 for handling and proofreading, 10 for proofreading by authors, 37 for make-up, 26 for printing of page proof, 1 for proofreading of page proof, and an estimated 20 for final printing.

Once again, I take great pleasure in expressing my gratitude to all who have helped maintain the standards of our Journal. In particular, I am indebted to the Editors for their critical evaluations and constructive suggestions; to the Business Manager and other officers of the Society for their advice and support; to Mr. John Ferguson, 3rd, and staff of the Monumental Printing Company for their skill and cooperation; to Mrs. Albert E. Dimond for indexing Volume 47; to all contributing authors for the opportunity of editing their manuscripts; and to the Sustaining Associates and the Rockefeller Institute for Medical Research for their continued financial support.

Finally, I wish to thank all Society members for the privilege of serving as Editor-in-Chief for 3 very rewarding years.

A. F. ROSS, *Editor-in-Chief*

Report of the Advertising Manager. Dr. L. J. Alexander retired as Advertising Manager on January 1, 1957, and thanks are due him and Mrs. Alexander for their valuable assistance to the present Advertising Manager and for having done the difficult work connected with advertising in Volume 47 of PHYTOPATHOLOGY.

Thirty-six pages of paid advertisements, 1.5 pages of *Phytopathological Classics* ads, and 4.5 pages of American Phytopathological Society News appeared in the first 6 issues of Volume 47. Financial statements are in the report of the Business Manager.

The co-operation of Mr. F. L. Brown, Monumental Printing Company, Dr. Saul Rich, Treasurer, and of Dr. A. F. Ross, Editor-in-Chief, is deeply appreciated.

D. A. ROBERTS, *Advertising Manager*

Report of the A.P.S. Representative, A.I.B.S. Board of Directors. The American Institute of Biological Sciences has operated 3 years as an independent organization incorporated under the laws of the District of Columbia. During this period of operation, there has been a steady growth in 1) biological societies working with the Institute, 2) services rendered the societies and individual biologists, 3) increase in responsibilities assumed in advising and administration of national and international biological problems, and 4) increase in budget administered by the Institute. The total unduplicated membership in the Institute has grown to just under 50,000.

The Institute has staged numerous conferences and committee meetings on biological problems. The Institute has assisted societies in securing travel funds for attendance at international meetings and helped societies in arranging for special meetings, publications and other services, as for example in relation to the American Phytopathological Society Jubilee Bicentennial Meeting in Bloomington, Indiana, August 24–28, 1958.

JAMES G. DICKSON, *Representative*

Report of the A.P.S. Representative, A.A.A.S. Council. I attended the meeting of the Council held at the New York meeting of the A.A.A.S. in December. Perhaps the most important vote passed by the Council at that meeting was to raise the dues of the A.A.A.S. from $6.50 to $8.50 per year. This is the first time that the dues of the A.A.A.S. have been increased for many years. According to the report given to the Council, the mean of an association with 20,000 or more members in the United States is about $10.00. This would mean that the $8.50 figure would be smaller than the mean.

Also, the Council approved a recommendation of the Board of A.A.A.S. to merge SCIENCE and SCIENTIFIC MONTHLY. This will be done in the January, 1958, issue.

The Council elected various vice presidents of the association, including Harry J. Fuller of the University of Illinois as Secretary for Section G, Botanical Sciences, and L. P. Reitz of the Agricultural Research Service at Beltsville, Maryland, as Secretary for Section O for Agriculture.

The Council also elected Wallace P. Brode as President-Elect of the American Association for the Advancement of Science.

JAMES G. HORSFALL, *Representative*

Report of the A.P.S. Representative, Agricultural Research Institute, N.R.C. Members of the Agricultural Research Institute were recently advised that the Sixth Annual Meeting will be held in Washington, D. C.

The Agricultural Board met May 1 and 2, 1957, and appointed an *ad hoc* organizing committee for a proposed International Conference on the Behavior and Mode of Action of Systemic Pest Control Chemicals in Plants. This group met with members of industry in February and strongly endorsed the conference but recommended postponement unless a minimum of $25,000 was pledged by April 1. Subscriptions were found not to be adequate, and the Conference plans for 1957 were cancelled.

The Subcommittee on Antibiotics and Systemic Pesticides also proposed to develop a bibliography on systemics and a treatise on the needs for systemic pest control chemicals.

The Subcommittee on Biological Control of Soil-borne Plant Pathogens proposed to develop an outline of methods in use for studying soil organisms as they may influence plant diseases. The significance of various techniques and the purpose served will be evaluated as applicable to the broad problem.

The latter subcommittee is participating, together with the other 5 subcommittees on insects, on nematodes, on plant diseases, on vertebrate pests and on weeds, in a project to evaluate economic losses caused by the respective pests. Selection for intensive study of specific crops or specific pests is being made. The Subcommittee on Plant Diseases finds it very difficult to collect reliable loss data.

This committee has decided to study some of the less well understood diseases and try to determine their significance and importance in crop production. One of the principal projects, beginning in 1957, will be a study of corn smut. Data on the incidence and location of smut on the plant will be correlated with losses in grain production. A study will also be made in the Palouse area in Washington on losses caused by wheat bunt, and in other areas there will be some studies on loose smut and wheat rust.

The Agricultural Research Institute continues to encourage and stimulate agricultural research, but it is evident that for it to do this effectively more financial support is needed.

C. L. LEFEBVRE, *Representative*
E. L. LECLERC, *Alternate*

Report of the A.P.S. Representative, Division of Biology and Agriculture, N.R.C. The annual meeting of the Division of Biology and Agriculture, National Research Council, met May 3–4, 1957, in Washington, D. C.

Topics of interest to Phytopathology were as follows: 1) The Biology Council was discussed, and it was recommended that the program of this organization be continued. 2) The booklet "Career Opportunities in Biology" is to be reprinted by the N.A.S.-N.R.C. 3) With respect to N.S.F. postdoctoral fellowships in Biology, it was felt that the quality of applicants was declining. As a member of the screening committee for these fellowships, this representative felt there were not enough plant pathologists applying. 4) A great deal of effort was expended by the staff of the

N.A.S.-N.R.C. in screening and placing scientists among the Hungarian refugees in 1956–57. 5) The proposal in 1956 for a Committee on the Utilization of Retired Scientists in Biology was again discussed but not acted on.

ST. JOHN P. CHILTON, *Representative*

Report of the A.P.S. Representative, Food Protection Committee, Food and Nutrition Board, N.R.C. The Food Protection Committee continues to devote its attention, for the most part, to intentional additives to food. The conference discussions and ensuing reports issued from time to time therefore are somewhat removed from the direct sphere of phytopathological research.

The report of the subcommittee on "The Practical Equivalent of Zero" presented at the December 13, 1956, meeting in Washington, D. C., deals with a problem of considerable interest to plant pathologists. The formation of this subcommittee was mentioned in last year's report. The work of the committee dealt with the problems posed by the possible presence in foods of trivial amounts of substances arising from the complex processing and packaging operations of the modern food industry. To designate such quantities as the "practical equivalent of zero" would, on the surface, appear to be a possible solution of the legal problems involved. Some extremely difficult problems arise, however, in attempting to devise a procedure for determining the "practical equivalent of zero." It is obvious that no general minimum level of concentration can be established as trivial, since compounds differ markedly in their toxicological properties.

The subcommittee submitted the following conclusions: 1) That the term "practical equivalent of zero" has no rigorous scientific basis, and that to designate a finite concentration as zero cannot be justified on a scientific basis (although it may be expedient legally to do). 2) That a "harmless" or "trivial" level of any substance in foods can only be determined on the basis of a quantitative study of its biological effects or, in other words, by the establishment of a tolerated level. 3) That the general principles involved in relating a minimum deleterious level to a harmless level deserve further careful consideration. (Perhaps such consideration by a group of pharmacologists and toxicologists could result in the development of a workable formula for deriving from the minimum level of observed toxicity a level at which the probability of damage would be so incredibly low as to approximate zero. The committee has in mind here a mathematical or statistical derivation rather than the present rule-of-thumb factor of safety.)

Dr. Paul E. Johnson, Executive Secretary of the Food Protection Committee, has called to the attention of the Committee the following 2 publications: 1) *Safe Use of Pesticides in Food Production.* Publication 470, National Academy of Sciences—N. R. S., Washington, D. C. Price $0.50. 2) *WHO Report on Toxic Hazards of Pesticides.* No. 114, WHO Technical Report Series 1956. Available at $0.60 from Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York. These 2 publications deal with topics of interest to phytopathologists who use pesticides in spraying and dusting operations.

J. C. DUNEGAN, *Representative*

Report of the A.P.S. Representative, Board of Editors, American Journal of Botany. The representative on the Board of Editors has worked with the editorial staff of the American Journal of Botany and reviewed various manuscripts when requested.

A. J. RIKER, *Representative*

Report of the A.P.S. Representative, American Type Culture Collection. A certified audit of the Collection's finances, December 31, 1956, showed assets of $99,111.25. This figure exceeds that for 1955 by $1892.38. This increase comes from an increase in the sale of cultures amounting to $672.00, a reduction in our annual rental charge amounting to $1200.00, and a larger bank balance from funds received as a research grant from the National Institutes of Health. The income from the sale of cultures in 1956 was $48,220.86. Culture sales for the first 6 months in 1957 were less than 1% under the sales during the same period in 1956.

The $7,848.72 which was remitted to the Collection by the National Research Council has increased to $8,420.27 as of December 31, 1956, from dividend credits. The original amount is the balance from the funds collected by the Council from industrial contributors. This growing fund is being held in reserve.

A research grant of $13,700.00 was obtained from the National Institutes of Health for 1 year. Depending on the availability of funds, $11,000 will be granted the second year, with the possibility of a smaller grant being made the third year. The terms of this grant are such that 15% of it ($2055.00) can be applied to general overhead. The remainder is used for salaries ($8650.00) of personnel working on the project, and for equipment, etc. ($2995.00). The project is on the preservation and taxonomy of the bacteriophages.

Another research grant of $2500.00 was obtained from the Office of Naval Research for 1 year only. This grant allows nothing for overhead expenses. It covers salaries to the amount of $1800.00, a refrigerator ($400.00) and supplies ($300.00). This project covers the preparation of a special reserve set of lyophilized specimens of important cultures in the Collection; these are to be stored in a fireproof building apart from the ATCC laboratory. We are finding this to be a rather expensive project because there is no allowance for the high overhead. There has been an unusually large amount of paper work connected with it.

Revision of the Collection's catalogue is in progress. The manuscript should be ready for the printer early in September. Owing to high printing costs, it has been decided to charge $1.00 per copy. Depending on changes in postal rates and regulations, this price will cover about 90% of the cost of the catalogue.

As indicated in the report for 1956, salary increases for the staff constitute a major financial problem. We have given some moderate increases this year, and we are paying for Blue Cross hospitalization and Blue Shield medical service for all the staff; however, our salary scale is about 20% below that of the present Federal Civil Service and from 10 to 12% lower than the scales in universities located in the Washington area. We are endeavoring to solve this problem, but we face difficulties. The Board members representing the Society of American Bacteriologists feel that a "head-tax" on members is not likely to meet with the approval of the membership. This plan was suggested by a member of that Society last year.

It is estimated that the income from an endowment fund of about $45,000 will solve the problem. If our NRC reserve fund and our public utilities stocks are taken as the starting fund (about $12,000) for such an endowment, it will be necessary to raise $33,000 to attain the estimated goal of $45,000. Research grants cannot be depended on for maintaining a proper level of salaries, as they are too uncertain from year to year.

Another problem we may have to face in the near future involves the expense of maintaining cultures for which there is little or no income from sales. Many of the plant pathogens are in this category. Although these cultures have scientific value, they are not profitable to maintain because most research pathologists maintain their own collections while engaged on a problem. The Collection has over 4000 cultures. For perhaps a fifth of these there is little or no consistent sales demand, and about 10% of the total are plant pathogens. Considering that we operate on such a narrow margin, it may become necessary to seek subsidies for the maintenance of these cultures.

H. H. MCKINNEY, *Representative*

Report of the A.P.S. Representative, Associates Food and Container Institute. The Activities Reports

of the Food and Container Research and Development work of the Quartermaster Food and Container Institute for the Armed Forces were received and studied by your representative. Most of their interest was concerned with canning, freezing, and dehydration of fruits and vegetables. Considerable work was also carried on looking toward overcoming food monotony and the preservation of better flavor for various commodities. The work on radiation preservation of fruits and vegetables still commands a high place in their program, and this is one of the most likely places that members of our Society can contribute something of value. Conferences have been held in which particular attention was given to the effects of irradiation on sprout control and on development of decay in potatoes.

Here at our laboratory we have continued our project on the "Control of Postharvest Diseases of Fruits and Vegetables by Radiation." Abstracts of 2 of our papers have already been published in PHYTOPATHOLOGY. Since that report, we have tried various dosages of gamma radiation on onions inoculated with Botrytis, carrots inoculated with Stemphylium, and sweetpotatoes inoculated with Rhizopus. At present, it appears that none of these commodities can withstand gamma radiation sufficiently to make it worth while trying to control the above pathogens in this manner.

It is recommended that a representative be appointed for 1958.

G. B. RAMSEY, *Representative*

Report of the A.P.S. Representative, Biological Stain Commission, Board of Trustees. American Phytopathological Society members during the current year have published at least 4 papers that describe new staining procedures for demonstration of causes of diseases. Three of these techniques apply to detection of viruses; one to differentiating morphologically similar species of the same fungus genus. To comply with recent discussions by the Biological Stain Commission it is recommended that, when possible, certified stains be used in these precise techniques and that the certification number be cited. This is necessary because stains and related chemicals manufactured in other countries are now generally sold in the American market. Many of these foreign stains, while the same in name, are different and cannot be certified within the standards set by the Biological Stain Commission. Therefore it is imperative that authors protect their recommendations by stating exactly what stains they used when establishing new or precise techniques.

F. P. MCWHORTER, *Representative*

Report of the A.P.S. Representative, American Standards Association Sectional Committee on Common Names for Pest Control Chemicals, K62. On May 1, 1957, arrangements were completed whereby the secretarial functions of the above sectional Committee, K62, including the processing of proposed American Standard Common Names, will be handled directly by the American Standards Association from their New York office. Mr. C. E. Hilton of the A.S.A. staff will be Secretary of K62. Formerly, Mr. J. A. Noone of the Agricultural Chemicals Association had performed these duties. Dr. H. L. Haller, U. S. Department of Agriculture, will continue as Sectional Chairman.

The Committee continues to pass on the suitability of proposed common names which are submitted from time to time by various sponsors. To date all sponsors have been industrial concerns with a direct interest in the pesticide. An application fee of $300.00 to defray expenses is required for each name submitted for consideration, and this is not refundable if the name fails to meet approval.

The Committee works closely with similar foreign organizations and during the year expressed comment on a list of 11 common names which the Canadian Standards Association is considering adopting. Some 40 additional common names are also under consideration by this group.

The Second Plenary Meeting of ISO/TC81, i.e. the International Standards Organization for Common Names for Pest Control Chemicals, will meet from September 18 to 20, 1957, at the British Standards House, London. At present there are 12 participating member countries and 20 observing member countries.

Continued representation by the American Phytopathological Society on A.S.A. Sectional Committee, K62, is recommended.

S. E. A. MCCALLAN, *Representative*
W. D. MCCLELLAN, *Alternate*

Report of the Committee on Extension. The entire committee has not met formally this year; however, several members have met informally, and correspondence contacts have been maintained. The committee sponsored an Extension Workers' Breakfast Meeting during the 1956 Cincinnati A.P.S. Meetings. This was attended by 44 persons including research, extension, and commercial personnel. The agenda included presentations and informal discussions of 1) better college curricula for future county agents and extension specialists, 2) the use and costs of color in extension publications, and 3) new methods of visualizing plant pathology.

Efforts and objectives of the 1957 committee include 1) improvement of undergraduate college curricula for extension workers, 2) preparation of color slide sets of vegetable, fruit, cereal, ornamental, and tree diseases for sale to extension and commercial agricultural workers, 3) investigation of the possibility of establishing a National 4-H Plant Disease award program, and 4) the preparation of an Extension Workers' Handbook for new extension pathologists.

The committee will sponsor a plant pathology workshop at the 1957 Stanford Meetings at which certain of the above items will be considered. In addition, an extra meeting of extension pathologists has been planned.

ARDEN F. SHERF, *Chairman*

Report of the Committee on Finances and Investments. Total invested funds $88,081.81 earned $3,358.48 in the year ending June 30, 1957, a yield of 3.81%. The yield last year was 3.56%.

The following table summarizes our investments and their earnings for the past fiscal year:

Investment	*Total*	*Earnings*	*Yield*
Insured Savings & Loan Accounts	$73,156.38	$2,547.83	3.48%
Common stock (original cost)	11,796.38*	716.73	6.07
U.S. Savings Bonds (at maturity)	2,925.00	87.75 (est.)	3.00
New Haven Savings Bank Account	204.05	6.17	3.02
Totals and average yield	$88,081.81	$3,358.48	3.81%

* *At the close of market July 9, 1957, our common stock was worth $18,400.63.*

During the year the following transactions were conducted: 1) $282.63 plus rights for 7 sh. of Johns-Manville common stock; 2) $213.14 plus rights for 2 sh. of Am. Tel. & Tel. common stock; 3) $9,000.00 Standard Federal Savings and Loan Account for 50th Anniversary Celebration reserve.

In order to get a ruling from the Internal Revenue Service concerning our tax status, a claim was submitted for refund of all past income tax paid by the Society on dividends and interest. The claim, for a total of $1,128.77, was filed on March 30, 1957. To date we have had no ruling from the Internal Revenue Service.

A. E. DIMOND, *Chairman*

Report of the Committee on History of Plant Pathology. The Committee on the History of Plant Pathology has maintained intact all items previously deposited and, insofar as time permitted, has attempted to put the file in order for use by those concerned with the subject in preparation for the 50th Anniversary Meeting of the Society. No outstanding acquisitions were made during the period under review.

J. A. STEVENSON, *Chairman*

Report of the Committee on International Cooperation. The principal activities of the Committee during this short year can be summarized as follows:

1) Dr. H. A. Rodenhiser and the Chairman conferred in February with Dr. J. A. B. Nolla, of Puerto Rico, also a Committee member and Chairman of the Comité Fitopatologia Latina-Americano de Cooperacion, regarding the activities of the special Committees of that organization and the ways in which cooperative regional tests of varietal resistance of crops to diseases, of mutual interest to Latin America and North America, could be effectively promoted. There has been progress.

2) Regional tests for varietal resistance have been started in various other regions, notably western Europe. It is hoped that the Committee may be useful, especially through FAO and certain other organizations, in encouraging and helping to coordinate these activities.

3) The Committee has continued to try to stimulate certain groups, especially in Latin America, to contribute useful summaries for the FAO PLANT PRODUCTION BULLETIN. There has been some success.

4) The Rockefeller Foundation, through Committee member J. G. Harrar, Director for Agriculture, has continued to be directly and indirectly helpful in promoting the objectives that are implicit in the Committee's mandate.

5) The Chairman of the Committee will attend the International Congress of Plant Protection in Hamburg in September and then study the status of plant protection in India. This will be an opportunity to confer with respect to ways in which the A.P.S. may be helpful.

6) It is the consensus that the A.P.S. might well accomplish something concrete by donating PHYTOPATHOLOGY to a carefully selected list of institutions in countries with poor dollar exchange. There is, however, strong sentiment for sharing our Jubilee Celebration by donating PHYTOPATHOLOGY to a certain number of needy institutions.

E. C. STAKMAN, *Chairman*

Report of the Committee on Phytopathological Classics. PHYTOPATHOLOGICAL CLASSICS' participation in the Society's Golden Anniversary celebration will consist of the publication of PC No. 10: Agostini Bassi's 2 classic papers on pathogenicity entitled "Del mal del Signo". A letter from Dr. G. A. Ainsworth, University of Exeter, who is making the translation from the Italian, states, as of July 12, 1957 that "The translation is finished and the Introduction drafted." Competitive bids are being solicited for the printing of PC No. 10 as well as for the reprinting of PC No. 3, the original edition of which is now exhausted.

The report of the Treasurer, Dr. Paul H. Wooley, for the fiscal year July 1, 1956, through June 30, 1957, is as follows:

Receipts:

Cash balance July 1, 1956			$1648.28	
Received during fiscal year 1956-57:				
From sale of CLASSICS		$157.25		
Originals	$135.50			
Reprints	21.75			
Accounts receivable 1956-57		28.25		
Originals	24.50			
Reprints	3.75		185.50	$1833.78

Disbursements:

Refunds on cancelled orders	$ 18.00	
Halftones for advertisements	43.05	
Postage	30.00	
Letterheads and stationery	14.50	
Cash balance on June 30, 1957	1728.23	$1833.78

The number of copies of PHYTOPATHOLOGICAL CLASSICS sold during the fiscal year was 182, with a total value of $185.50. Numbers of CLASSICS on hand in Ithaca, New York, and in Lake Alfred, Florida, on June 30, 1957, were as follows: No. 1, 481; No. 2, 383; No. 3, 0; No. 4, 89; No. 5, 312; No. 6, 359; No. 7, 295; No. 8, 476; No. 9, 690.

L. C. KNORR, *Chairman*

Report of the Committee on Phytopathological Monographs. The Committee on Phytopathological Monographs has considered the desirability of a uniform outline for monographs. The committee agreed that no specific outline should be made mandatory for all authors, since it would not be possible to apply a single uniform outline to all subjects. Therefore, a general suggestion of the range, scope, and arrangement of the monographs has been made to prospective authors.

At present 9 monograph topics have been accepted by authors and are under preparation. Other authors for selected topics are being contacted. One monograph is nearly complete. It is hoped that several will be ready for sale at the time of the Golden Anniversary. No contacts have yet been made with publishers, since no manuscripts are ready for submission.

One manuscript was offered to the committee dealing with regional history of plant pathology. It was the view of the committee that our charge from the Society did not include historical material, and the manuscript was not accepted. It is believed that the Council should consider the matter of proper referral of such manuscripts.

G. C. KENT, *Chairman*

Report of the Committee on Phytopathological News Page. Through the excellent cooperation of members comprising the Standing Committee for preparation of the News Page in PHYTOPATHOLOGY, we received items in sufficient number to complete the alternate one page or one-half page reserved for NEWS during the past year. The Editor wishes to acknowledge his appreciation to members of the Committee for their prompt and efficient relaying of news items. During the past year the Committee consisted of the following: L. O. Weaver, College Park, Maryland; Shirl O. Graham, Pullman, Washington; D. E. Ellis, Raleigh, North Carolina; S. H. Davis, New Brunswick, New Jersey; Roy D. Wilcoxson, St. Paul, Minnesota; and M. W. Cormack and E. J. Hawn, Lethbridge, Alberta, Canada. Dr. Cormack accepted a new position in June, 1957, and felt that his greater responsibility would no longer permit him to serve as the Canadian representative on the Committee. He suggested E. J. Hawn as an alternate, and Dr. Hawn kindly consented to complete the remainder of Dr. Cormack's term. The Editor and members of the Committee would appreciate receiving suggestions concerning style of presenting items or any changes that might make the NEWS page more attractive or stimulating to the membership.

K. W. KREITLOW, *Chairman*

Report of the Committee on Phytopathological Reviews. In one sense the program to stimulate increased interest in and production of review articles in plant pathology has been less successful than might have been hoped in the 7 months since the establishment of the Committee. In another sense it has been overly successful. As Chairman, I should like to take this opportunity to thank the other members, Dr. Ralph Shay and Dr. W. H. Sill, Jr., for their support and patience during the past year and to absolve them of any responsibility for the decision to move slowly in the matter of soliciting review articles for the Society journal.

The Committee has devoted much time to assembling a series of suggested topics and to a consideration of their relative merit. The list has 66 topics. It would be helpful to have ideas from members.

As the program of the 50th Anniversary Committee developed, it became apparent that we would have to be cautious lest duplication of effort take place, and we are grateful to Dr. G. S. Pound for keeping us informed as his program took shape. Nevertheless, as is inevitably the case, this information came in only very gradually and I have taken it upon myself to delay the Reviews program until the Anniversary series is decided upon in its final form. If, as Dr. Shay suggests, there are more review articles prepared in conjunction with the Anniversary program than can be published in the jubilee volume, it may prove wise to have the Reviews committee consider them for publication in the regular journal of the Society.

There is what seems to be an increased interest in phytopathological reviews on the part of the ANNUAL REVIEWS organization. Quite possibly this is a result of our agitation of a year ago but in any event is to be welcomed, provided that we can avoid duplication of effort. In any event, Dr. Horsfall has been appointed a member of the editorial board of the ANNUAL REVIEWS OF PLANT PHYSIOLOGY, with special responsibility for articles on plant pathology. He has already notified me of 3 such articles that he is suggesting for the next issue.

There are, as of the moment then, several groups actively interested in the solicitation of review articles in plant pathology: the BOTANICAL REVIEW, ANNUAL REVIEWS OF PLANT PHYSIOLOGY, for the time being the 50th Anniversary Program, and the A.P.S. Reviews Committee. As a member of the Anniversary group, Dr. Shay can effectively maintain liaison between the jubilee program and the Societies Reviews Committee. As Advisory Editor for plant pathology on the BOTANICAL REVIEW board, I can similarly see that no duplication occurs here. I would like to recommend that Dr. Horsfall be added to the Standing Committee on Phytopathological Reviews, not to replace any of the present members, but to afford convenient liaison with the ANNUAL REVIEWS organization. I think it entirely possible that a beneficial exchange of views and material among the 4 outlets can develop under this arrangement; at the very least, duplication of effort can be minimized.

The Reviews Committee last year was to explore further the matter of a bibliography of review articles, annotated, to be put out probably as a special issue of PHYTOPATHOLOGY and supplemented from time to time thereafter with shorter lists appearing in the regular journal. I would like to request authorization to prepare and submit a proposal, in a form satisfactory to the Reviews Committee, to the President of the Society, and to the Editor-in-Chief of the journal, for a grant from N.S.F. to prepare an annotated bibliography of review articles.

R. B. STEVENS, *Chairman*

Report of the Committee on Placement. Forty plant pathologists had applications in the active files of the Placement Committee during 1957. Nine persons were placed, and 31 are currently being considered for positions. The minimum salary requested by applicants ranged from $4000 to $15,000. Three of the applicants were women. Age of applicants ranged from 26 to 55 years, and all except 7 were citizens of the United States.

Applications were sent to 35 prospective employers in an attempt to fill 44 positions. These positions were of the following kinds: 12 with state agricultural experiment stations or universities, 7 with the U. S. Department of Agriculture, 4 with other federal agencies, 4 with teachers colleges, 4 foreign assignments, 1 with an arboretum, and 12 with commercial firms. From 1 to 8 applications were submitted to each prospective employer; the total number of applications mailed was 196.

Persons interested in federal positions should fill out Standard Form 57 and mail it to the U. S. Civil Service Commission, as it is becoming increasingly difficult to place persons in federal service unless their names are included on a Civil Service roster.

It is the responsibility of the applicants to keep the Committee informed of changes in address or employment status. If after 3 attempts the Committee does not receive a reply from an applicant his application is placed in the inactive files. All records and files are currently up to date.

E. W. HANSON, *Chairman*

Report of the Committee on Public Relations. The work of this Committee continued along the lines initiated 3 years ago, namely the publicizing of some of the outstanding plant disease research papers. A series has been prepared and published in the major farm magazines. In addition, our writer, Robert Rathbone, has made possible the presentation of certain phases of our pathological activities through official channels. Our Committee has assisted the Committee on Teaching of Plant Pathology in the preparation of a career brochure. Press releases for about 15 papers that seem to have publicity value will be issued at the time of their presentation at Stanford. Excellent coverage of material published in PHYTOPATHOLOGY has resulted through the courtesy of the Editor-in-Chief, who has made galleys available 2 months prior to publication.

The effectiveness of our program would be materially improved if more of our Society members would participate by sending copies to us when their articles are accepted for publication, since timing is an important factor in public relations work.

PAUL R. MILLER, *Chairman*

Report of the Committee on Regulatory Work and Foreign Plant Diseases. Two sessions of the Committee were held during the Cincinnati meetings of the Society. The first was on Thursday evening, December 6, 1956, after the Society's General Symposium on Foreign Plant Quarantine and Regulatory Administration. The second was held jointly with the Advisory Committee on Nematology and the Committee on Seed and Plant Material Certification, on Saturday morning, December 8.

The symposium had been arranged at the request of the Committee by its Chairman, James G. Dickson, who presided. Members of the Committee attended.

The following items were discussed and acted upon at the first session. 1) The Committee recognized the need for a plant nematologist in its membership and recommended to the Society Council that Gerald Thorne of the University of Wisconsin, formerly nematologist for the U. S. Department of Agriculture, be appointed to the Committee. 2) The Committee recognized the need for an up-to-date compilation of information on foreign plant diseases and recommended to the Council that the United States Department of Agriculture give consideration to a revision of John A. Stevenson's *Foreign Plant Diseases, A Manual of Economic Plant Diseases Which Are New To Or Not Widely Distributed in The United States*, published in 1926. This manual has not been revised since its original publication. The Committee had been informed that a considerable amount of new information on foreign plant diseases is available. 3) After considerable discussion on the hazards of seed-borne plant pathogens coming into the United States on imported seeds, the Committee recommended to the Council that the Society approve in principle and recommend that the U. S. Department of Agriculture consider the laboratory examination of imported seeds for the purpose of detecting seed-borne plant pathogens. 4) Since the legislation proposed in Senate Bill No. S3952 of the Eighty-Fourth Congress, which previously was approved in principle by the Committee and by the Society, had failed to become enacted into law before the Congress adjourned, the Committee recommended to the Council that the Society give similar approval to a proposed new bill for the Eighty-Fifth Congress for enactment of this legislation. The legislation would enable the U. S. De-

partment of Agriculture to regulate the importation and movement of plant pathogens. The Department already has authority to regulate the importation and movement of insect pests under the Federal Insect Pest Act of 1905.

At the second session of the Committee, held jointly with the 2 other Committees, consideration was given primarily to problems concerning certification of seeds intended for export and import for freedom from plant pathogens and nematode problems. The following were pointed out:

1) Better communication is needed between countries in regard to their respective requirements and the procedures that need to be followed in the matter of treatments and permits.
2) Some seeds are coming into the United States without any inspection.
3) So little information is available on distribution of species of plant parasitic nematode that it is difficult for regulatory officials to make a decision as to acceptance or rejection of an importation when inspection shows nematodes to be present. Survey knowledge concerning nematode species in the United States is needed.
4) Pathogens have variable composition and characteristics as to species, races, and forms, particularly with respect to biological forms within a species. Information needed to determine and support regulatory action in specific cases often is lacking.
5) Production of pathogen-free propagating material at source is one of the best approaches to the problem.

It was the consensus, both in relation to the nematode problems and in relation to the problems of seed certification for freedom from plant pathogens, that the best procedure for the present is to continue to seek and obtain the needed information; to follow conservative methods in the meantime; and to endeavor to develop clean-up treatments. It also was the consensus that an atempt should be made to state these problems in a concise item for publication in the NEWS page of PHYTOPATHOLOGY as an expression of the Commmittee on Regulatory Work and Foreign Plant Diseases.

GILBERT L. STOUT, *Chairman*

Report of the Committee on Seed and Plant Material Certification. The emphasis of the Committee's work during this year has differed somewhat from that of preceding periods. Methods for obtaining and maintaining pathogen-free planting material, basic and essential to any certification program, are of primary interest. The legal procedures for handling such healthy material, being derivative, are reserved for future study. The program is to prepare a summary on each group of crops, along the lines of that for ornamentals in PLANT DISEASE REPORTER SUPPLEMENT 238. Each member of this Committee is chairman of a subcommittee that will study a crop group. Because of the recency of this new program, there is little progress to report.

The Committee is cooperating with the International Seed Testing Association Committee on Plant Diseases. They are starting a series of brief monographs on diseases caused by seed-borne pathogens. Specialists are being sought who will prepare such statements on individual diseases. This program is complementary to that of this Committee.

K. F. BAKER, *Chairman*

Report of the Committee on Fungicide Colloquium. A Fungicide Colloquium program was, unfortunately, impossible to arrange for this year. The usual notice regarding placement of the program in the Annual Meeting program was not received. By the time deadline information for programs was received, it was too late to arrange properly for the type of program which has become an important feature at the Annual Meeting.

The Committee has given some comments and suggestions on the program being arranged for the 50th Anniversary Program. In deference to the nature and scope of the Anniversary Program, it is recommended that the Colloquium as such be suspended for 1958.

Since the Colloquium has reached a recognized and established status as part of the Annual Meetings of the Society, it is recommended that the Fungicide Colloquium Committee be kept intact and that provisions be made for a place in the program for the Colloquium at the Annual Meeting in 1959.

L. GORDON UTTER, *Chairman*

Report of the Committee on Industrial Relations. During the past year, several meetings of portions of the Industrial Relations Committee have been arranged. The principal problems have been to discuss and implement means of increasing participation of industry members in Society activities, mainly from a scientific standpoint, and to develop a program for securing maximum contributions for support of the Golden Jubilee Anniversary celebration in 1958.

With regard to the first item, all research organizations doing work in the field of phytopathology were contacted. Those who have members in the Society were encouraged to present scientific papers at either the Palo Alto or the Bloomington Meeting.

Since the problem of securing contributions of the order of magnitude requested by the Jubilee Committee will be a difficult task, the Committee believed it necessary to have a clear insight into the nature of the program and the use to be made of the funds which are solicited. Industry is called upon so often by various organizations for contributions that, unless our story can be made very convincing, we are doubtful of success. The outline of the program and the proposed budget have been furnished us, and formal solicitation will be commenced after the Palo Alto Meeting.

We plan to contact as many industrial organizations as possible at Palo Alto to lay the groundwork for a formal request which will be mailed later. These plans will be completed at Palo Alto.

E. E. FEICHTMEIR, *Chairman*

Report of the Committee on Membership. As Chairman of the Membership Committee of A.P.S. for 1957, I wrote to each committee member on February 1 and made assignments by states as indicated below. Each committee member was asked to designate 1 person at each institution in his area to serve as a contact to solicit new members. Follow-up letters have been written to committee members to encourage active solicitation.

In view of our report's being required at the end of only 7 months' activity and before we can reach the crop of graduate students that will enroll in September, the number of new-member applicants for this period will be small. Each member of the committee has been very cooperative in accepting his assignment. The committee memberships and their territories are as follows:

Robert Campbell (Minnesota, Montana, North Dakota, South Dakota, Wyoming)
W. J. Hooker (Michigan, New Jersey, Ohio, Pennsylvania, Connecticut, West Virginia)
D. A. Roberts (Delaware, Maine, Maryland (Incl. Beltsville), Massachusetts, New Hampshire, New York, Vermont)
R. G. Grogan (Arizona, California, Idaho, Nevado, Oregon, Washington)
Arthur Kelman (Alabama, Florida, Georgia, North Carolina, South Carolina, Virginia)
J. E. Thomas (Arkansas, Louisiana, New Mexico, Oklahoma, Texas, Mississippi)
J. E. Kuntz (Colorado, Iowa, Kansas, Nebraska, Utah, Wisconsin)
P. M. Simmonds (Canada)
J. R. Shay (Illinois, Indiana, Kentucky, Missouri, Tennessee)

GLENN S. POUND, *Chairman*

Report of the Committee on Teaching of Plant Pathology. The brochure on career opportunities in plant pathology is in the final stages of preparation before being given to the publications office. With good luck, it should be out in January.

Dr. Wm. B. Hewitt, as Secretary of the Society, passed along a suggestion from the Council that the Committee on Public Relations through its chairman Dr. Paul Miller could be of assistance in preparing the final copy. Consequently, the material is now in the hands of the Public Relations Committee for study and revision. Photographs of pathologists at work are needed.

A suggestion from Dr. George Kent, Cornell University, that our committee consider setting up an exhibit on teaching of plant pathology at the Bloomington meeting has been received and acted on. Insufficient time existed between receipt of this suggestion and preparation of this report to contact committee members. It is the opinion of the chairman that this would be an excellent plan, and Dr. McCallan is being sent a request for space allocation.

For the meeting in 1959, the chairman hereby requests permission to organize a panel discussion, or other suitable program, on teaching of plant pathology for 1 evening. Numerous requests for such a session have come in during the last several years. Several more departments now have curricula for undergraduates in plant pathology than had them when such curricula were last discussed at an annual meeting. This could be one of the topics for discussion. If and when authorization is given for organizing a program on teaching of plant pathology, the chairman stands ready to function.

W. H. BRAGONIER, *Chairman*

Report of the Committee on Virus Type Culture Collection. Owing to the short interim between the reports for 1956 and 1957, we cannot show much tangible progress in the plant virus antiserum project. Definite plans for a preliminary program in cooperation with the Microbiological Research Foundation, Bethesda, Md., have been made. The Foundation is injecting rabbits with the purified antigens as they are received from the contributors. The resulting antisera will be supplied to the contributors for verification tests against the respective antigens to determine the validity and practicability of the whole procedure. The outcome of this study, the problems encountered by the Foundation, and the demand for antisera will determine the next step to be taken.

The following purified antigens have been either supplied or promised: potato X virus, A. F. Ross; tobacco mosaic virus, H. H. Thornberry; tobacco ringspot virus, Russell L. Steere; alfalfa mosaic virus, Russell L. Steere; Cymbidium orchid mosaic virus, A. H. Gold; Odontoglossum orchid mosaic virus, A. H. Gold; barley stripe mosaic virus, M. K. Brakke and Ellen Moorhead; brome grass mosaic virus, M. K. Brakke and Ellen Moorhead.

It is of interest to note that the Microbiological Research Foundation is exploring the field of virus culture in plant tissue cultures.

The work of revising the Catalogue and Registry of the Plant Viruses is in progress.

H. H. MCKINNEY, *Chairman*

RESOLUTION ADOPTED BY THE COUNCIL ENDORSING THE PROPOSED PROGRAM OF THE SUBCOMMITTEE ON VIRUS TYPE CULTURE COLLECTION FOR THE PRODUCTION AND DISTRIBUTION OF ANTISERA TO BE USED FOR THE RAPID IDENTIFICATION OF PLANT VIRUSES

Whereas plant virus diseases, which are caused by a large number of virus species and strains, are of great economic importance and rapid dependable methods of identification are essential in virus research, in crop-improvement programs, in certification programs, and for the indexing of important plant materials passing through quarantine,

And whereas serological methods have been demonstrated to provide rapid, dependable method for the identification of viruses attacking many crop plants,

And whereas antisera are stable, noninfectious entities that can be exchanged freely among workers in different areas and in different countries,

And whereas the facilities in the United States for the purification of virus antigens and for the production of antisera are meager in relation to the needs,

And whereas the Subcommittee on Virus Type Culture Collection has given serious thought to the problem and has proposed 1) that the Subcommittee initiate a trial project for the production of antisera by an institution that is prepared to produce on a commercial scale, 2) that these antisera be placed in the hands of competent virologists for adequate testing before they are distributed, 3) that these antisera be prepared with viruses deposited in the American Type Culture Collection or that will be so deposited, and 4) that suitable standards for all procedures involved in the production of antisera ultimately be set up by the Subcommittee,

Be it resolved, therefore, that the American Phytopathological Society through its Council endorse this proposed program and lend its support to it wherever possible,

And be it further resolved that copies of this resolution be sent to such individuals as deemed desirable by the Subcommittee.

Report of the Advisory Committee on Collecting and Disseminating New Fungicide Data. Dr. A. G. Newhall, Department of Plant Pathology, Cornell University, was responsible for the entire program relating to this project. Publication of the results, as in previous years, was in AGRICULTURAL CHEMICALS. The *Results of 1956 Fungicide Tests* appeared in the February, March, April, May, June, July, and August, 1957, issues of this magazine. The considerable amount of tabular material, and high costs relating to same, necessitated serial distribution of the results in over twice the number of issues previously involved. Also, the cost of reprints has gone up since 1956 from 15¢ to 16⅓¢ per copy, which is in line with increased labor costs. The total number of reprints has been kept to 1500 in order to reduce large storage requirements.

As indicated in our previous report, all activities in handling the project such as orders, mailing, etc., were centralized at Dr. Newhall's office. Following his departure on sabbatical leave January 1, 1957, Dr. D. A. Roberts and Miss Dorothy Parkin continued the administrative and routine details of the project.

Single copies of reprints, which should be available shortly, will be mailed gratis to the companies originally underwriting the project.

Publicity relating to the availability of the reprints is being arranged for in the PLANT DISEASE REPORTER and AGRICULTURAL CHEMICALS. Notices will appear in SCIENCE, CROPLIFE, A.I.B.S. BULLETIN, JOURNAL OF ECONOMIC ENTOMOLOGY, JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, BETTER FARMING METHODS, CROPS AND SOILS, AMERICAN POTATO JOURNAL, AMERICAN FRUIT GROWER, AND PHYTOPATHOLOGY.

Since the reprints have not been distributed, no report can be made on the number of orders and sales at this time. The Summary Report from Dr. Saul Rich, Treasurer, on the status of project as of August 6, 1957, is as follows:

Total receipts	$2300.00
Total sales	1441.85
Total available	3741.85
Total expenditures	2453.25
Balance on hand	1288.60

The fund is $230.75 under the balance reported as of November 12, 1956, which would appear to indicate a slow depreciation of our original reserve. Our costs for reprints for the 1956 results will be $244.00 and that, plus mailing, secretarial help, correspondence, etc., will exceed the estimated income of $400 for anticipated sales of reprints at $1.00 per copy. As yet it does not seem possible to bring

this project to a completely self-sustaining financial basis. Consequently, after this year an increase in the price of reprints may be necessary.

In our last report, reference was made to the desire of Cornell to terminate this project. Consultation with Drs. Newhall, Roberts, Kent, and others indicated that they would be unable to continue this work. The Committee appreciated their fine cooperation and efforts during several years and honored their request to have the project transferred to another institution. After considerable deliberation, it was agreed that Dr. A. B. Groves, Virginia Experiment Station, Winchester, Virginia, would be a most logical and acceptable choice to handle the project. Dr. Groves accepted, and arrangements were made for transference of the work, reprints, correspondence, and records to Winchester on July 15. Dr. Rich was also requested, as approved by Council last year, to transfer the funds for this project to Dr. Groves. This fund will be set up as an American Phytopathological Society Fund in Dr. Groves' bank. He will have signature status to withdraw funds, and an alternate signature will be arranged for Dr. Rich or his successor as Treasurer of the Society. A financial statement will be made annually to the Treasurer of the Society. Dr. Groves will solicit, receive, and collate the data on new fungicide tests, prepare the manuscript for publication, administer the handling and filling of orders, and handle other matters incidental to the project. The Committee will assist him in any manner that may be possible. The Chairman has met with Dr. Groves and gone over many of the problems involved. We consider it fortunate to have a man of Dr. Groves' experience and background take over this work.

L. Gordon Utter, *Chairman*

Report of the Advisory Committee on Integration of Research on Soil-Borne Pathogens. In spite of the fact that the membership of this Committee is geographically widely scattered and international in scope, the Committee met once during the year. An added international flavor was attained, in committee, by holding part of its meeting in a third country.

Activity of the Committee is reflected in 3 significant moves it has made during the year. First, it instigated interregional and international representation at the March joint session of the Technical Committees for the Western Regional Projects W-12 and W-38, which deal with soil-borne pathogens. Second, it has been instrumental in arranging for at least interregional representation at the joint meeting in October of the Technical Committees for the Southern Regional Projects S-26 and S-35. And third, through representation on the subcommittee that is planning the Soil Microbiology symposium for the 50th Anniversary meeting, it has participated in obtaining exceptionally broad international representation for this important meeting.

The Committee also has considered additional means of integrating research in the field of soil-borne pathogens through the exchange of techniques and information between research groups and individuals here and in Canada.

The Committee is convinced of the desirability of fostering integration of research in this important and rapidly growing field of plant pathological soil microbiology and of bringing about exchanges of personnel and data at various meetings. The Committee is aware that this is true not only of this but of all areas of research. But it is also aware that, unless it is the responsibility of some individual or committee to foster such integration, little is actually accomplished.

W. C. Snyder, *Chairman*

Report of Elections, Appointments, and Other Business

The ballots for the annual election were counted by the Council on the evening of August 25. Members of the Council on the ballots were excused for the counting. The following were elected by a majority of the votes cast: *President-Elect*, Glenn S. Pound; *Vice President*, W. C. Snyder; *Councilor-at-Large*, Roderick Sprague.

At the general business meetings, the Society approved the recommendations of the Council appointing the Representatives and Committees as listed above and, for 3-year terms beginning January 1, 1958, Kenneth F. Baker, Stephen Diachun, Charles L. Fergus, David Gottlieb, and Marr D. Simons as Editors of Phytopathology.

The Society also approved proposals 1) that the membership fee to A.I.B.S. for 1958 be on the basis of $1 per member (as done in 1956 and 1957) rather than the $1000.00 maximum required and 2) that the $1500 budget provided the Standing Committee on Public Relations be continued for 1958.

Future Meetings

The 50th Anniversary Meeting will be held on the campus of the University of Indiana, Bloomington, Indiana, August 24-28, in conjunction with the A.I.B.S. This, our Jubilee Semicentennial Meeting, will bring scientists from various countries of the world to take part in symposia covering fields of interest to all plant pathologists and many fields of biology.

The 1959 meeting will be held with the A.I.B.S. on the campus of Pennsylvania State University during the first week of September. This meeting follows the IX International Botanical Congress at Montreal, Canada.